The role of roadside ditches as conduits of fecal indicator organisms and sediment to downstream drinking water supply systems

by Kimberly Falbo

THE ROLE OF ROADSIDE DITCHES AS CONDUITS OF FECAL INDICATOR ORGANISMS AND SEDIMENT TO DOWNSTREAM DRINKING WATER SUPPLY SYSTEMS

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ABSTRACT

Bacterial and sediment pollution is widespread in US waters, contributing to increases in human disease as well as aquatic ecosystem degradation. Identifying pathways of pollutants from source to stream may help improve water quality management. Few studies have examined the impacts of roadside ditch networks on water resources, though ditches are ubiquitous. The goal of this study was to determine if roadside ditches are conduits for fecal indicator organisms and sediment and if land use, specifically manure amendment, affects these concentrations and loadings. Seven roadside ditches were monitored for *Escherichia coli* (*E. coli*) using ISCO™ automated water samplers and the Idexx Colilert™ system, as well as total suspended solids, pH, conductivity and flow for one year in central New York. Ditches were either adjacent to manure amended agricultural fields or predominately forested land. *E. coli* concentrations in ditch water samples following storms ranged from undetectable to >241,960 MPN/100mL and frequently exceeded NYS DEC and US EPA recommendations. Overall, ditches adjacent to manure amended fields had significantly higher concentrations and loads of *E. coli* than forested sites, though this was dependent on the season. The concentrations were also unexpectedly high in the forested sites, with possible sources including wildlife, pets, septic wastes and livestock. Peak concentrations were observed in both summers following manure spreading with declining levels thereafter, but viable organisms were detected throughout the year. Viable *E. coli* were also present in ditch sediment between storm events and therefore were available for resuspension and transport. Total suspended solids concentrations reached as high as 52.2 g L^{-1} and were overall significantly higher for agricultural sites as compared to forest sites. There was a complex association between total suspended solids and *E. coli* concentrations. These findings gain significance when placed in the broader framework that roadside drainage networks are acting to rapidly shunt stormwater runoff to downstream drinking water supplies. As a result,

recommendations to reduce pathogen transport and improve water quality should focus on reducing farm runoff, using buffer strips or constructed wetlands and improving roadside ditch management.

BIOGRAPHICAL SKETCH

Kimberly Falbo was born and raised in Rochester, NY. She received a BS in Natural Resources from Cornell University in 2007. She then worked as the project manager for the Healthy Home, a home environmental health education organization. Kimberly returned to Ithaca in 2008 to start her graduate degree.

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INTRODUCTION

Bacterial water pollution can lead to unsafe drinking water, restrictions on recreation opportunities and closures of shellfish beds. According to the United States Environmental Protection Agency (US EPA), pathogens are the leading cause of impairment to 303(d) listed water bodies with pathogen contamination affecting almost 25% of impaired waters (US EPA, 2010). Sources of pathogens and associated indicator organisms include humans, domesticated animals and wildlife and may enter waterways from urban stormwater, combined sewer overflow systems, septic systems, wastewater treatment plants and animal feeding operations (Smith & Perdek, 2004). While sources are diverse, land use is a strong predictor of degraded bacterial stream water quality, specifically with urban, urban-agriculture and agriculture associated with higher levels of degradation than forest and urban-forest (Francy, Helsel, & Nally, 2000). However, there are still many unknowns in the processes that link pathogen sources to downstream waters.

Manure amendment is a critical source of pathogens and associated indicator organisms. The United States has an estimated 350 million tons of animal manure produced each year (Smith & Perdek, 2004). Many studies have documented elevated levels of fecal indicator organisms in agricultural fields and in waters downstream from where manure amendment or livestock grazing have occurred (Niemi & Niemi, 1991; Patni, Toxopeus, & Jui, 1985). In a Nova Scotia stream, water downstream from a dairy farm had the highest percentage of samples exceeding water quality standards for irrigation and recreation. This stream reach was responsible for approximately 45% of the fecal coliform load reaching the watershed outlet, while concentrations in the headwaters were below 5 MPN/100 mL (Jamieson, Gordon, Tattrie, & Stratton, 2003). Simon and Makarewicz (2009) analyzed non-event stream samples with different land management practices in Conesus Lake sub-watersheds in western NY. The sub-watershed with active grazing of cows and manure amendment had the highest monthly loading per hectare for 4 out of 5 years when compared to agriculture with little or no manure amendment and was higher than a predominately forested site for all years.

While manure amendment is a strong overall predictor of fecal indicator organisms, weather conditions, specifically high precipitation and snowmelt, have been shown to increase concentrations of pathogens. Outbreaks of waterborne illness due to drinking water contamination have been associated with large precipitation events, e.g. those exceeding the 90^{th} percentile in the preceding two months (Curriero, Patz, Rose, & Lele, 2001). In addition to disease, levels of indicator bacteria have been found to be elevated during times of high precipitation when compared to baseflow in the Hoosic River in Massachusetts, the Buffalo River in Buffalo, NY and in tributaries to drinking water reservoirs in Germany (Kistemann, et al., 2002; Pettibone & Irvine, 1996; Traister & Anisfeld, 2006). The majority of the fecal indicator organisms entering downstream of dairies also occurred during storm events (Jamieson, et al., 2003; Robert D. Simon & Joseph C. Makarewicz, 2009).

Fecal indicator organisms are often bound to sediment, which affects organism transport processes. Elevated levels of bacteria during storms may be due to the movement of organisms attached and free floating in overland flow as well as the resuspension of bottom sediment. Past research has found a strong correlation between fecal coliform and total suspended sediment concentrations (Mallin, Johnson, & Ensign, 2009; Pettibone & Irvine, 1996) and between *E. coli* and turbidity (R. D. Simon & J. C. Makarewicz, 2009). However, the level of association between sediment and fecal indicator organisms is varied and somewhat uncertain. Proportion of fecal indicator organisms attached to particles ranged from as low as 9% to as high as 44% (R. C. Jamieson, D. M. Joy, H. Lee, R. Kostaschuk, & R. Gordon, 2005; Muirhead, Collins, & Bremer, 2006a; Oliver, Clegg, Heathwaite, & Haygarth, 2007). The fraction of attachment also varied between baseflow and stormflow conditions, with between 20 to 35% attached to settable solids during baseflow and between 30 to 55% attached during stormflow (Characklis, et al., 2005). Muirhead et al. (2006b) concluded that fecal indicator organisms that are attached to sediment are transported at lower rates and therefore are maintained in the system. Once in the system, they can survive in streambed sediment for up to 6 weeks and were found to be resuspended during the rising limb of storms (R. C. Jamieson, D. M. Joy, H. Lee, R. Kostaschuk, & R. J. Gordon, 2005). Synthesis of these studies suggests that management practices to reduce soil erosion and movement will likely decrease pathogen transport as well.

A critical question is the length of time that organisms remain viable and the factors that affect survival. If the organisms remain viable in the soil environment for long periods after deposition, there will be more opportunities for organisms to be transported and contaminate water. Extreme temperatures, extreme pH, moisture, nutrient supply and solar radiation are the most important factors controlling the survival of enteric bacteria in the soil and water environment (Crane & Moore, 1986). A review by Oliver et al. (2005) found survival of fecal coliform and *E. coli* outside of the host ranged from 7 to 630 days and depended on whether it was deposited in water or soil, the associated environmental conditions, and whether the feces remained intact or was applied as manure/slurry. *E. coli O157:H7* in Dutch soils under different agricultural management was detectable for an average of 80 days with a range from 54 to 105 days (Franz, et al., 2008). The interaction between survival and retention processes caused 80 to 90% of the *E. coli* applied to the land to remain in the soil column (Fenlon, Ogden, Vinten, & Svoboda, 2000). While die-off may reduce the number of organisms, survival in the soil is longer than the recurrence interval of precipitation events in most non-arid environments, making transport a key target for management.

Determination of the pathways from fields to streams is imperative for improved bacterial water quality. Roadside ditches are ubiquitous and designed specifically to efficiently move water away from the road surface, yet their role in water pollution conveyance has largely been ignored. Roads are a dominate feature in the United States landscape. It is estimated that approximately 15 to 20% of the US is impacted ecologically by roads, with rural roads impacting a much larger area than urban roads (R. T. T. Forman, 2000; R. T. T. Forman & Alexander, 1998). Riitters and Wickham (2003) found that across the conterminous US, 50% of the land area is within 382 m of a road and increases to 97% if the distance is expanded to only 5000 m. Where there are roads, there is road drainage, as this management is critical to protect road function and reduce costs. Precipitation floods roads, causes potholes and cracking and reduces roadbed stability when it is saturated (Richard T. T. Forman, 2003). These effects cause premature failure of roads and can be costly to highway departments. Approximately 25% of highway agency budgets in New York are spent on drainage (Orr, 2003).

Unintended consequences of this effective drainage have received little attention by water resources managers or researchers in agricultural landscapes. Past research has focused on the hydrologic impact of roads in the western United States, especially in areas logged. Roadside ditches have been found to carry a large amount of water, not only from the road surface but also from the landscape. In a small watershed in central New York, approximately 25% of the landscape was draining to the roadside ditch network before ultimately draining to a stream (Rebecca Schneider, personal communication, unpublished data). A study conducted in Oregon on forest roads found that approximately 14% of the landscape was draining to the roadside ditches and then to a culvert. Within each culvert's sub-basin, 95% of the flow was not from the road surface, but from subsurface interception (B. C. Wemple & Jones, 2003). Roadside ditches can be connected to streams both directly and indirectly. For example, 34% of the road segments had roadside ditches draining directly to the stream, while 24% had ditches draining to a culvert, which had a gully down to the stream (B. Wemple, Jones, & Grant, 1996). Hydrologic connection of roads to streams ranged from 15 to 57% (Mills, Dent, & Cornell, 2007; B. Wemple, et al., 1996). These connections can cause changes to the hydrologic regime in streams. Roads have been shown to increase channel density by capturing and routing surface and subsurface flow to streams and decrease contributing area required for channel initiation (Richard T. T. Forman, 2003; Montgomery, 1994; B. Wemple, et al., 1996). The peak discharge increased by 20% and initiation of the storm hydrograph advanced by 10 hours in a watershed in the western Cascades after roads, though high variability did not lead to statistical significance (Jones & Grant, 1996). On the other hand, a modeling study on roads without roadside ditches in northern Thailand saw only a 2 to 5% increase in peak discharge when compared to the same land use scenario without roads (Cuo, Giambelluca, Ziegler, & Nullet, 2008).

The focus of water quality monitoring from road runoff has been on sediments, heavy metals and deicing agents (R. T. T. Forman & Alexander, 1998). Sediment erodes from the surface of the road, cutbanks, fillbanks, near bridges and culverts and from ditches. They are carried through the ditches and the finer sediments are deposited into the streams (R. T. T. Forman & Alexander, 1998). In addition to finer sediments, roadside ditches also carry significant volumes of bedload and deposit them as deltas at the ditch's intersection with a stream (Diaz-Robles, 2007). Herbicides, insecticides, pesticides, nutrients and heavy metals, including lead, zinc, copper, chromium, cadmium, aluminum, iron, manganese, titanium, nickel and boron have also been found in road runoff (Coffin, 2007; R. T. T. Forman & Alexander, 1998; Trombulak & Frissell, 2000). Deicing agents are also well documented as being transported via roadside ditches (Coffin, 2007; Diaz-Robles, 2007; R. T. T. Forman & Alexander, 1998; Trombulak & Frissell, 2000). Though road runoff has been monitored in the past, fecal indicator organism transport via roadside ditches has not been documented.

Agricultural landscapes have largely been left out of the recent US EPA stormwater regulations. Phase I of the US EPA stormwater program focuses on municipal separate storm sewer systems in urbanized areas with more than 100,000 people. Phase II expanded regulation coverage to include urbanized areas with more than 50,000 people and non-urbanized areas if the population was at least 10,000 with a population density of at least 1000 people per square mile (United State Environmental Protection Agency, 2005b). Densely populated urban areas (those with at least 50,000 people and 1000 people per square mile) cover approximately 2% of the landscape (United State Environmental Protection Agency, 2005a). At the same time, bacterial contamination from manure amendment is not regulated directly, as the National Pollution Discharge Elimination Scheme only regulates manure when it is stored (Ferguson, Husman, Altavilla, Deere, & Ashbolt, 2003). Stormwater regulations have ignored rural areas, even though they are a larger portion of the landscape and have land management practices that are high risk for bacterial pollution.

The **overall goal of this study** was to investigate the role of roadside ditches as conduits of fecal indicator organisms from agricultural fields to downstream drinking water supply systems. Specific objectives were (a) to monitor the concentrations and loads of fecal indicator organisms in roadside ditches associated with manure-amended fields during and following storm events as compared with the same parameters in roadside ditches adjacent to forest, and (b) to determine if fecal indicators survive in the roadside ditch bottom sediment between storms providing the potential for resuspension during the next flow event.

METHODS

Study Site Description

This study was conducted across a 1600 km^2 area in Cortland and Cayuga Counties, central New York, US. A total of seven roadside ditches were monitored within four watersheds (Figure 1). Watersheds 1, 2 and 3 are all sub-basins of the Cayuga Lake watershed, which drains north to the Great Lakes Basin. Surface water from the basin is the drinking water supply for the towns of Dryden, Ithaca, Seneca Falls and Lansing, the villages of Cayuga, Aurora, Seneca Falls, Lansing and Cayuga Heights and part of the City of Ithaca and serve almost 90,000 people (Genesee/Finger Lakes Regional Planning Council & EcoLogic, 2000). Watershed 4 is a sub-basin of the Susquehanna River Basin. There are over 335 public water supply systems in New York that lie within the Susquehanna River Basin, drawing from both surface and ground water (Susquehanna River Basin Comission, 2008). Central New York has a temperate climate with average minimum temperatures of -10^{o}C in January and February and average maximum temperature of 27^{o}C in July and August. Average annual precipitation is 94 cm and average annual snowfall is 173 cm. Predominate soil types include Lordstown, Mardin channery sit loam, Volusia channery silt loam, Erie channery silt loam, Langford channery silt loam, Valois-Howard gravelly loam, Bath and Valois gravelly silt loam and Chenango gravelly loam in Watershed 1 and 2. Predominate soil types in Watershed 3 and 4 include Honeoye silt loam, Palmyra gravelly loam, Angola silt loam, Ovid silt loam, Lima silt loam and Lansing gravelly silt loam (USDA Natural Resources Conservation Service, 2010). The land use in Watershed 1 and 2 is approximately 4% developed, 58% forest, 30% agriculture and 6% wetlands, while the land use in Watersheds 3 and 4 is approximately 4% developed, 17% forest, 72% agriculture and 5% wetlands according to the National Land Cover Database.

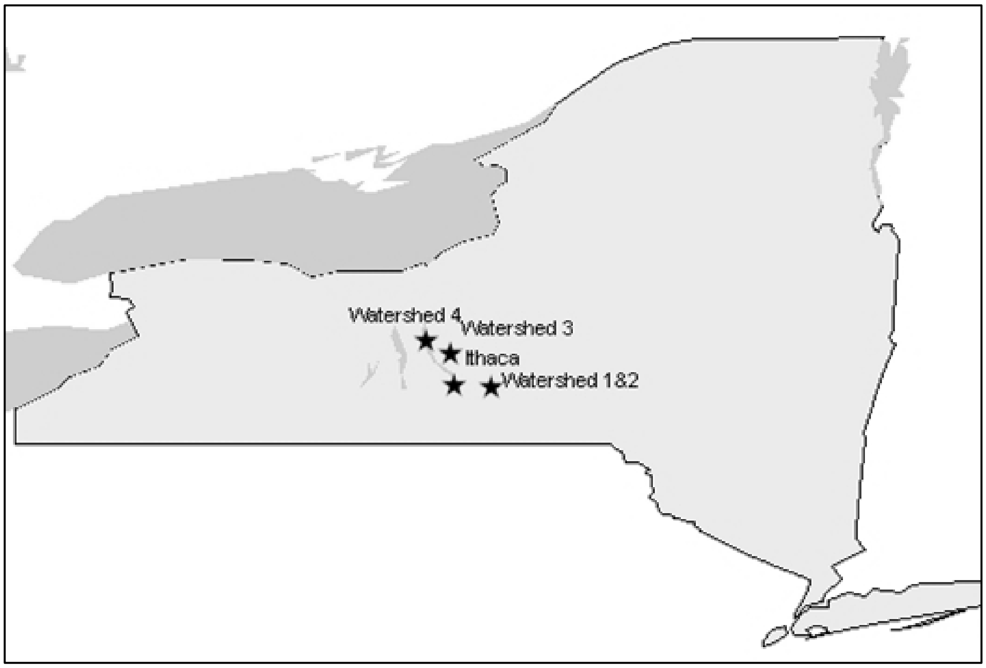


Figure 1. Sampling site watersheds.

Roadside Ditch Monitoring Stations

Roadside ditches were selected for monitoring if they met four basic criteria. First, all ditches had contributing areas with one predominate land use, manure-amended agriculture or forest, as determined by on-the-ground visual surveys supplemented with aerial imagery from Google Earth©. The forested sites acted as a reference, indicative of background levels, and enabled us to determine the relative contributions of pollutant transport in roadside ditches from agriculture compared to forest land use. It was difficult to find ditches with only forest in the contributing basin, so it should be noted that there was a residence in the basin for Ditch 1. Second, all ditches had a vegetated bottom. Ditch management, specifically the extent the ditch bottom is vegetated, has been shown to strongly influence the amount of sediment moving in the roadside ditches (Diaz-Robles, 2007). It was important to eliminate this as a confounding factor. Third, the landowner, either a producer or resident, and town highway staff granted permission for us to monitor their ditch. Although roadside ditches are in the town's right of way, we sought landowner and community support. Last, monitoring sites were limited to ditches connecting directly into the stream network to accommodate a parallel study on the hydrologic effects of roadside ditches. Because few ditches were adjacent to only one land use and community politics made farmers hesitant to be involved in water quality research, the first seven ditches identified that met the selection criteria were monitored (Table 1).

Table 1. Site description for roadside ditches selected for monitoring, central New York, USA.

Ditch Number	Watershed	Land Use	Produced Samples	Basin Area m^2	Length m	Depth m
1	1 - Virgil	Forest	Yes	95545	220	0.46
2	2 - Owego	Ag	Yes	22341	175	0.28
3	3 - Salmon	Ag	Yes	21512	176	0.39
4a†	3 - Salmon	Forest	No	46933	232	0.40
4b	3- Salmon	Forest	No	33370	189	0.59
5	4 - Paines	Ag	Yes	145844	205	0.75
6	2 - Owego	Ag	No	41023	234	0.98
7	4 - Paines	Forest	Yes	116448	236	0.36

† Ditch 4a was equipped for 2008, while Ditch 4b was equipped for 2009.

The sampling set-up was the same for all monitoring sites. Each ditch was equipped with an Isco Automated Water Sampler™ (Model 6712, Teledyne Isco, Lincoln, NE), which was powered by a marine battery and a 5-watt solar panel. The water intake with a strainer and the Isco Liquid Level Actuator™ (Model 1640, Teledyne Isco, Lincoln, NE), which initiates sampling when water is detected, were placed in the deepest part of the ditch approximately three to four centimeters from the bottom. Each water sampler was filled with 12 1-L high-density polyethylene bottles that were either new or were acid washed in 10% hydrochloric acid for 4 hours, rinsed three times and autoclaved. One gallon buckets of frozen water were placed inside each sampler prior to each storm event to keep the holding temperature of samples below 10^{o}C. In addition, each roadside ditch also was equipped with a Trutrack WT-HR Water level/Temperature Datalogger™ to monitor the water stage. For protection from sedimentation, the Trutrack was placed into a window screen-covered 3.8 cm PVC pipe with 1 cm holes. The PVC pipe was buried so that the zero mark was 10 cm below the ditch surface to monitor soil saturation.

The Northeast Regional Climate Center has weather monitoring stations in both geographic regions of the study. These weather stations were within 15.2 km of our ditch monitoring stations. To ensure the weather stations were representative of our sites, we equipped each ditch monitoring station with a manual Tru-Chek™ rain gauge.

Ditch Discharge Determination

Water discharge rates within each ditch were determined using rating curves and continuous water stage measurements. The velocity was measured along a transect perpendicular to the direction of flow at an average of three locations, with increasing numbers of measurements for wider ditches. At each velocity measurement location, the water depth and the location along the transect were measured to calculate the cross-sectional area. The water flow was then calculated by Equation 1, where width is the midpoint between two consecutive sampling locations:

$$discharge\left({}^{m^3}\!/_{s}\right) = \sum\nolimits_{measurment} velocity\ ({}^{m}\!/_{s}) * width\ (m) * depth(m)$$

Equation 1. Determination of discharge from velocity measurements.

A rating curve was created for each ditch relating the water stage (depth) to the discharge at the time of measurement. The continuous flow was then calculated from the rating curve equations and the continuous water stage measurements.

Rating curve development was complex due to culverts and environmental conditions. The velocity measurements were combined over the entire sampling period, as the ditches were flashy and it was difficult to get high flow measurements through all the seasons. The rating curves had coefficients of determination that ranged between 0.6456 and 0.9595 (Table 2). Some adjustments were made to several of the rating curves. For Ditch 1, velocity measurements from a large storm in March 2009 had low water stages but high water flows and were not representative of flow at other times. This may be caused by an error in the instruments, lack of vegetation, or frozen ground. These values were not included in the rating curve. Though this ditch did have a culvert at the bottom, it was assumed that this did not affect the discharge and loading measurements. The ditch and the culvert had approximately the same capacity and no backflow was ever observed. The maximum water stage reading was 348 mm, while velocity measurements were recorded at 242 mm. Thirteen of 228 bottles had water stage measurements above 242 mm, but only seven were more than 10 mm above 242 with a maximum of 106 mm. For Ditch 3, it was assumed that the Trutrack was shifted during the winter months, as multiple feet of snow were piled on top of it. The average difference between the observed depth and the measured depth of water was 8.44 cm before winter in 2008 and was 12.26 after winter in 2009. The water stage measurements for 2009 were adjusted in both the rating curve equation and the flow calculations by 3.82 cm, the difference between the average observed and measured depths between the two years. For Ditch 5, the maximum depth with a velocity measurement was 427 mm, while the maximum water stage was 1092mm. This ditch had a culvert, which appeared to be restricting flow and causing backflow to occur. For all stages above 427mm, we conservatively estimated the flow to be the same as that at 427 mm stage. For all other ditches, rating curve stage measurements were as deep or deeper than those used for the loading calculations and therefore did not impact the results. Actual rating curves are included in Appendix A.

Table 2. Equations relating water stage in mm to water flow in m^3s^{-1} for roadside ditches.

Ditch	Rating Curve Equation	R^2
1	10^{-17} Stage$^{6.2536}$	0.9137
2	10^{-5} $e^{0.033 \text{ Stage}}$	0.9478
3	$3 * 10^{-18}$ Stage$^{6.8186}$	0.6456
5 For stage < 427 mm For stage >427 mm	10^{-11} Stage$^{3.878}$ 0.16986	0.7605
7	$4 * 10^{-7}$ $e^{0.0487 \text{ Stage}}$	0.9595

Water Sample Collection

Because the majority of roadside ditches only contain water during and following storms and snowmelt, samples were only collected after a rainfall and/or snowmelt event. It was considered a storm event if there was enough rain to produce flow in at least one of the roadside ditches being monitored. For the summer and fall sampling seasons, every event was monitored except during one two-week period in August. During the spring 2009 field season, samples were collected at two-week intervals due to the unreasonable high frequency of sampling events.

Samples were collected automatically at pre-programmed time intervals during an event. From June 2008 to August 19, 2008, 12 samples were collected per event. The first six samples were collected every 30 minutes and the last six samples were collected every 1.5 hours. Due to processing times and no substantial changes to *E. coli* and TSS concentrations in the later portions of the storms, the sampling configuration was changed to seven bottles. The first five bottles were collected at 30 minute intervals, the 6th bottle was collected after an additional 2 hours and the 7th bottle was collected after an additional 3 hours. A total of 648 water samples were collected and processed throughout the entire study.

Bottles within the automated water samples were open and were sometimes in the field for weeks before an event occurred. For each ditch and each event with fewer than 12 bottles collected, one empty bottle was collected from the field to determine if storage and handling techniques contaminated the samples. One hundred milliliters of sterilized, deionized water was added to each field blank in the lab, shaken and then processed for *E. coli*. Of the 80 field blanks tested, 7 had detectable *E. coli* concentrations. All concentrations were less than 8.4 MPN/100mL, so it was assumed that bottles were not significantly contaminated while sitting open in the samplers.

Because the methods used in this study detected viable *E. coli*, the holding time and holding temperature of the water samples were important. According to *Standard Methods for the Examination of Water and Wastewater* (Section 9060B, Eaton, Clesceri, Rice, & Greenberg, 2005), nonpotable water for compliance purposes is required to be held below 10^{o}C for a maximum of 6 hours of transport and 2 hours of refrigeration, while water for noncompliance purposes is required to be held below 10^{o}C for a maximum of 24 hours. It was however impossible to process samples within 8 hours of collection because: 1) we collected water samples over the course of the entire storm event (7.5 to 12 hours), some of which occurred during the night, and 2) it took about 5 hours to retrieve the samples from the sampling sites. A study by Pope et al. (2003) sought to determine if longer holder times and higher temperatures, which is often the case when completing field research, would significantly alter *E. coli* densities using Colilert® and Quanti-tray®/2000. They concluded that samples held at 4°C and 10°C did not show significant decreases in *E. coli* densities until they had been held for at least 48 hours. Though samples held at 20°C showed significant decreases in 2 of 4 sites when held at 24 hours, *E. coli* densities did not show significant decreases if held 8 hours or less (Pope et al. 2003). Therefore, it was assumed that if samples were held for less than 48 hours at less than 10°C or if samples were held for less than 8 hours at up to 20°C, holding time and temperature did not significantly affect the results. When sample holding time and temperature did not meet these criteria, it was assumed that the *E. coli* concentrations were a conservative estimate, as all samples exceeding these criteria showed a significant decrease (Pope, et al., 2003). The temperature was recorded for the first bottle in each sampler at the time of collection, as it would be held within the sampling unit the longest and therefore would have the highest temperature.

Twenty one out of 103 bottles tested had a temperature above 10°C, though all were less than 20°C and 19 were less than or equal to 13°C. The maximum temperature was 19°C in Ditch 5 on July 17, 2008 and was taken soon after the sample was collected. On the other hand, 11 of the 103 bottles tested had a temperature less than or equal to 0°C due to cold outside temperatures. In addition to holding temperature, the holding time was also calculated. The average time a bottle remained in the field was 14.51 hours. Sixty bottles were in the field for longer than 24 hours and for these samples, the average bottle was held for 27.11 hours. The maximum holding time was 44.18 hours, which was still less than the 48 hour threshold. Fourteen of the 103 samples had holding temperature above 10°C and were held for longer than 8 hours. Therefore, approximately 14% of the samples may be a conservative estimate of the *E. coli* concentrations.

Sediment Sample Collection

Sediment samples were collected at approximately two week intervals. Because the objective was to determine if *E. coli* was viable within roadside ditch bottom sediment between rain events, samples were only collected if there had not been a sampling event in the last 5 days. Sediment samples were collected using a modified version of the pvc pipe method; a sediment core was collected using a 1” diameter syringe with the top removed, which allowed easy transfer of the sample to the sample bottle. Each sample was taken a random number of paces (maximum of 9) upstream of the water sample intake. At each sampling site, 2 sediment cores, each 9.8-mL, were collected from the top 2 cm of sediment. One was placed in a 125 mL bottle for *E. coli* analysis. The other was wrapped in pre-weighed aluminum foil and placed into a plastic bag for moisture content analysis. Between each sample, syringes were rinsed, saturated for 1 minute with 10% bleach solution and rinsed with ultra-pure water. Each syringe was then placed in the soil at the sampling location prior to sample collection to ensure that no residual bleach would kill the *E. coli* in the sediment.

Storm Water Sample Processing

<u>*E. coli* Quantification</u>

E. coli is a fecal indicator organism and was used in this study to determine if there was the potential for water contamination with pathogens. To determine the concentrations of *E. coli*, “the Enzyme Substrate Coliform Test” was performed (Eaton, et al., 2005, Method #9223) using Idexx’s Colilert® and Quanti-Tray®/2000. Colilert® contains ortho-nitrophenyl-β-D-galactopyranoside (ONPG), which is metabolized by the total coliform enzyme β-D-galactosidase to produce a yellow color. Colilert® also contains 4-methylumbelliferyl-β-D-glucuronide (MUG), which is metabolized by the *E. coli* enzyme β-glucuronidase to produce fluorescence. This method quantifies viable total coliform and *E. coli* concentrations and was ideal due to the short processing time required for each sample. Analysis was completed for both undiluted samples and at a 1:10 dilution, except for two sampling dates where a manure odor was apparent and the samples were diluted to 1:20 and 1:100. The sample processing followed the Eaton, Clesceri et al. (2005) methods, except collection bottles were shaken vigorously before removing the 100mL for testing to ensure all sediment was resuspended. In addition, the trays were incubated at 37^{o}C. For dilutions, 10 mL of sample was measured using a 10 mL graduated cylinder, which was rinsed 3 times with ultrapure water between samples. To ensure this method was not transferring bacteria between samples, the graduated cylinder was filled with ultrapure water between bottles 3 and 4 for each ditch, diluted to 100mL with sterilized, deionized water and analyzed for *E. coli*. Of the 52 lab blanks analyzed, only 3 had detectable levels of *E. coli*. Two lab blanks had concentrations of 2 MPN/100mL or less. The lab blank concentration from Ditch 2 on May 29, 2009 was 193.5 MPN/100mL, when water sample concentrations exceeded the detection limit of 48,392 MPN/100mL. It was assumed that the dilution methods did not contaminate samples, as *E. coli* concentrations were almost always undetectable.

Cumulative Impacts of the Roadside Ditch Networks

The loads from an individual roadside ditch are likely to be diluted out when they enter a stream, since an individual ditch only carries a small portion of stream flow. The concern is the cumulative impact of the roadside ditch network and the goal is to gage whether this network could contribute large enough loads to lead to a detectable degradation of stream water quality. Therefore, a simplified model was developed to determine the cumulative load from the entire roadside ditch network within each watershed. This load was divided by the average stream discharge to determine the stream concentrations should roadside ditches be the only contributors of *E. coli.* Then, the number of days when sampling occurred that stream water *E. coli* concentrations exceeded the US EPA's least stringent bacterial water quality recommendation, 575 MPN/100mL, was computed.

The first step was to determine the cumulative load of *E. coli* traveling through the roadside ditch network. This required the determination of total length of roadside ditches within each watershed, but mapping was beyond the scope of this study. However, the roadside ditch network in Watershed 4 was mapped as part of a parallel study and was found to have a ditch density of 0.001875 m/m^2 (Brian Buchanan, personal communication, unpublished data). In addition, the road density within each watershed was determined using GIS. The ratio of roadside ditch density to road density for Watershed 4 was 1.63 and was assumed to be similar in the other watersheds, as this was the best available data. The roadside ditch density in the other watersheds was calculated by multiplying this ratio by the road density. Next, the average load of *E. coli* per meter of roadside ditch for each land use on each sampling day was calculated. This was aggregated across all ditches, because only one watershed had water quality data from both land uses and land management practices were assumed to be similar across watersheds. Finally, based on GIS layers from the National Land Cover Database, the land area for agriculture and non-agriculture was determined for each watershed. Cumulative load traveling through the ditch network was estimated by the following equation:

$$Cumulative\ load = \frac{5}{7} \times \sum_{land\ use} ditch\ to\ road\ density\ ratio\ \times road\ density\ \left({}^{m}\!/_{m^2}\right) \times land\ \ area\ (m^2) \times average\ load\ per\ meter\ ditch\ length\ ({}^{value}\!/_{m})$$

The load was multiplied by 5/7 to account for the fact that not all roadside ditches had flow.

The next step was to estimate the stream discharge in each watershed. Watersheds 1, 2 and 3 had USGS stream gages on them in the past, and the average daily flow was calculated for each season using all years of available data. Watershed 4 did not have a USGS stream gage, but was monitored as part of the parallel study on roadside ditch impacts on hydrology (Brian Buchanan, personal communication, unpublished data). Monitoring did not occur during February and early March, so the spring discharge calculations may be an undersestimate. With these average seasonal daily flows, the stream concentrations were calculated by dividing the daily total load from the roadside ditch network by the average stream flow.

Total Suspended Solids

Total suspended solids was determined using "Total Suspended Solids Dried at 103-105^{o} C", from *Standard Methods for the Examination of Water and Wastewater* (Eaton, et al., 2005, Method#2540D). The Millipore™ AP40 glass-fiber filter and the Millipore™ Chemical Duty Vacuum/Pressure Pump (115 v, 60 Hz) filtration system were used. First, the glass-fiber filter disks were prepared. Each was placed on the vacuum apparatus, rinsed three times with 20 milliliters of ultra-pure water, dried in a drying oven at 103 to 105^{o} C, placed in a desiccator for at least 15 minutes and weighed. The filter was then placed on the filtration apparatus and wetted to hold it in place. The original 1-L bottle of sample water was shaken to resuspend all solids and ensure the concentration was representative. Volumes ranging from 10 to 450 mL were then filtered. If the sample appeared to have a high TSS concentration, a small volume was filtered to ensure the filtering did not take more than 10 minutes. If the sample appeared to have a low TSS concentration, then a high volume was filtered to ensure the change in weight was large enough to detect. Once the sample had fully passed through the filter, the filter was rinsed three times with 10 mL of ultrapure water. The vacuuming continued for three minutes after all the rinses were completed. The filters were then dried for at least 1 hour in a drying oven at 103 to 105^{o}C, placed in a dessicator for at least 15 minutes and weighed. The concentration of total suspended solids was then calculated from the following equation (Equation 2):

$$total\ suspended\ solids, {}^{g}/_{L} = \frac{[(weight\ of\ filter + solids, g) - (weight\ of\ filter, g)] * 1000}{sample\ volume\ filtered, mL}$$

Equation 2. Calculation for total suspended solids concentration

<u>pH</u>

Sample pH was measured in the laboratory using a Denver Instrument™ Model 250 pH, ISE, Conductivity Meter with the Denver Instrument™ pH/ATC electrode. The pH meter was standardized daily with pH 4, 7 and 10 buffer solutions. It was rinsed with ultrapure water and blotted dry using a Kim-wipe prior to each measurement. The probe was placed in the sample water and the pH was read when the meter was at the lowest temperature reading for the sample and was stable for at least 10 seconds. The measurements were taken within an average of 32 days from the sample retrieval date, although exact holding times were not recorded for 175 of the 636 samples. The maximum holding time was 120 days. The long holding time was due to frequent field sampling, lengthy processing times and complications with a broken meter.

<u>Conductivity</u>

An Orion model 122 Conductivity Meter and an Orion 012210 Conductivity Cell, which automatically correct for temperature, were used to determine the conductivity. The probe was placed in a standard solution of 100 µs/cm weekly to ensure proper functioning. First, samples were shaken. Then, the probe was placed in the sample and a reading was taken as soon as the reading held constant for 10 seconds.

Ditch Sediment Analysis

E. coli Concentrations

The Colilert® and Quanti-Tray/2000 method was used to determine the relative concentrations of *E. coli* among the monitored roadside ditches. First, approximately 40 mL of sterile, deionized water was added to each sediment sample, so the total volume, including the sediment and the water, was 50 mL. The bottle was then shaken vigorously for 20 seconds to break up the soil core. The bottle sat for approximately 10 minutes to allow the largest particles, which could interfere with the quantification methods, to settle. Next, 20 mL of the water/sediment solution and 80 mL of sterile, deionized water were poured into a new bottle and was then processed the same as the water samples.

Sediment Sample Moisture Content

The sediment moisture content was determined using the "Thermogravimetric Using Convective Oven-Drying method" from *Methods of Soil Analysis Part 4-Physical Properties* (Dane & Topp, 2002). Samples were stored completely sealed to prevent water loss in the refrigerator until weighing could occur. Samples were weighed using a Mettler Toledo AT216 Delta Range Analytical Balance. The aluminum foil was punctured a few times to allow the water to escape and then samples were dried overnight at 103 to 105^{o}C. Each sample was placed in a desiccator for temperature stabilization and then reweighed. The water content was calculated using the following equation (Equation 3):

$$\% \, water \, content = \frac{[(wet \, sediment + tin \, foil \, weight, g) - (dry \, sediment + tin \, foil \, weight, g)] * 100}{dry \, sediment \, weight, g}$$

Equation 3. Determination of the % moisture content for sediment samples.

Statistical Analysis

JMP® statistical software was used for data analysis. The *E. coli*, TSS and conductivity concentrations, daily discharge, daily discharge depth, daily *E. coli* load and daily TSS load were not normally distributed and therefore were log transformed for analysis. Of the 629 water samples analyzed, 11 were below the detection limit for *E. coli* of 1 MPN/100mL, while 22 samples were above the detection limit. The concentrations were recorded as 0.5 MPN/100mL for samples below the detection limit and were kept as the detection limit when they were above the detection limit. For the sediment *E. coli* analysis, 9 of the 190 samples were below the detection limit of 1 MPN/100 mL and were included in the statistical analysis as 1 MPN/100mL. Thirteen of the 190 samples were above the detection limit and were included in the statistics as the detection limit. These samples were included in the statistical analysis to ensure the extreme highs and lows, which would have management implications, were represented.

RESULTS

Sampling stations were installed between late May and late June 2008. Due to a particularly dry June, sample collection did not begin until mid-July 2008 and continued until July 2009. Only five of the seven roadside ditches carried enough flow for sample collection, so water analyses were limited to five rather than seven ditches. A total of 648 water samples were analyzed for water quality parameters, though insufficient volumes sometimes lead to samples being analyzed for only 1 parameter. Discharge was monitored continuously for one year. Water samples were collected on 54 days over the course of the study. The number of days does not necessarily reflect the number of separate precipitation/runoff events, as flow within the ditch often continued beyond one calendar day. Sampling did not occur in both regions of study for each collection date. In Region 1 (Ditches 3, 4, 5 and 7) and Region 2 (Ditch 1, 2 and 6), samples were collected on a total of 32 days and 48 days, respectively, with sample collection occurring in both regions on 26 of those days. For seasonal analysis, the start of summer was defined by the first day of manure spreading that occurred in any of our study sites, which corresponded to April 22 in 2008 and April 14 in 2009. Spring was defined as February to the start of summer and fall was defined as October 1 to November 16. The roadside ditches were frozen during the winter, e.g. November 16 to February, so no samples were collected during that season.

Precipitation

Total rainfall associated with each sampling date was calculated by summing the rainfall in the 24 hours preceding the first sample collection in that region to the time of the last sample collection on that date for that region. On consecutive sampling dates, precipitation in the 24 hours preceding sample collection on day 2 was already accounted for in day 1's total rainfall. Any precipitation that was included in the previous day's total was removed to prevent overestimations of precipitation necessary to initiate sampling. The resultant minimum precipitation values were less than one millimeter (Table 3). Though sample collection did not always occur on the same day, there was no statistical difference between the mean precipitation in the two regions overall (p=0.1887) or by season (Spring: p=0.3231, Summer: p=0.1025, Fall: p=0.7111).

Table 3. Precipitation from the 24 hours preceding and during sample collection from Northeast Regional Climate Center weather stations in both sampling regions.

	Region 1 Precipitation (mm)				Region 2 Precipitation (mm)			
	Mean	SD	Range	n	Mean	SD	range	n
Overall	9.15	9.44	0.00 – 33.78	32	11.20	10.52	0.00 – 38.40	48
Spring	6.31	5.32	0.00 – 17.78	12	7.67	8.60	0.00 – 28.10	12
Summer	9.47	9.50	0.00 – 32.51	14	13.58	9.31	0.40 – 34.20	23
Fall	14.10	14.35	0.00 – 33.78	6	10.25	13.49	0.00 – 38.40	13

Discharge

Discharge was analyzed as the volume of flow over the course of one sampling day. Overall, spring had significantly greater volumes of flow than did summer (F-ratio=6.76, p=0.0016). For the agricultural roadside ditches, there was no significant difference in daily flow among the seasons, while spring and fall volumes were significantly greater than summer volumes for the forested sites. Overall, the agricultural sites had significantly higher flow than the forested sites (Table 4). This pattern was observed for both spring and summer, while there was no difference in fall daily flow between the adjacent land uses. Relative to the other ditches, Ditch 5 had the largest flow volumes over the course of the study.

To determine if the discharge results were dependent on the contributing area to each ditch, the discharge was also analyzed as a depth (volume/contributing basin area). The agricultural sites had significantly higher depths than forest roadside ditches overall and over each season (Table 4). There was no seasonal difference in discharge depth in the agricultural ditches, though summer was significantly lower than spring and fall for the forest ditches (Table 5). Ditch 5 and Ditch 2 had significantly higher depths overall than did Ditch 1 and 7, with no significant difference from Ditch 3 and the other ditches (Figure 2).

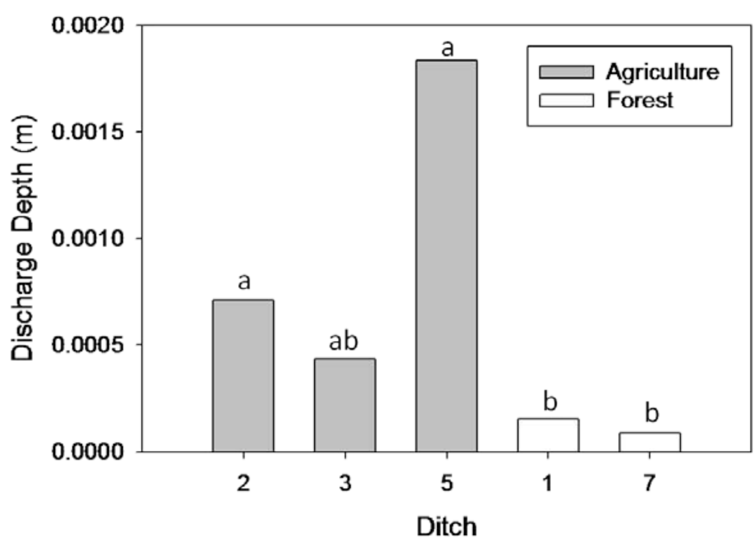


Figure 2. Geometric mean daily discharge depth for each roadside ditch. Different letters signify statistical differences at α=0.05 according to the analysis of variance test.

Table 4. Summary of the water and sediment sample concentrations by land use. Statistical significance was determined using a t-test on the geometric means, except pH was analyzed using arithmetic mean, and is denoted as * = 0.05, **=0.01, ***=0.001

	Agriculture					Forest				
	Arithmetic Mean	**SD**	**Geometric Mean**	**Median**	**Range**	**Arithmetic Mean**	**SD**	**Geometric Mean**	**Median**	**Range**
Discharge										
Volume*** -----m^3/day-------	370	839	52	38	0 - 4550	58	113	13	18	0 – 726
Depth*** -------mm/day------	3.1	5.6	1.0	1.3	0 .0– 31.2	0.6	1.0	0.1	0.2	0.0 – 6.2
Water Concentrations										
*E. coli**** ---MPN/100mL----	7,568	33,104	499	770	<1.0 - >241,960	1,481	3,114	210	248	<1.0 - >24,196
TSS*** --------g L^{-1}--------	0.9600	3.7797	0.0380	0.0290	0.0000 – 52.217	0.0452	0.1807	0.0063	0.0048	0.000 – 2.4623
pH***	7.83	0.38	------------	7.86	6.85 – 8.67	7.58	0.32	------------	7.57	6.66 – 8.56
Conductivity*** --------µS/cm-------	423.8	339.2	352.0	370.0	83.5 – 2370	291.5	148.6	258.1	265.0	68 – 1266
Water Loading										
*E. coli*** $1x10^9$ MPN/day	15.16	45.53	0.25	0.23	0.0 – 274.60	0.44	0.9	0.04	0.04	0.0 – 4.34
TSS*** ---- 10^3 g day^{-1}----	1241	4692	1.6	0.57	0.0 – 31211	1.3	5.3	0.1	0.07	0.0 – 40.6
Sediment										
E. coli* ---MPN/100mL---	382	688	48	52	<1.0 – 2420	265	668	27	20	<1.0-2420

Table 5. Summary of the seasonal arithmetic means for water parameters. Statistical significance was determined using the analysis of variance test (a>b>c at a 0.05 significance level within an individual land use).

	Agriculture			Forest		
	Spring	Summer	Fall	Spring	Summer	Fall
Discharge						
Volume ----m^3/day----	385±767a	521±1105a	136±211a	108±174a	15±25b	56±64a
Depth ------mm/day------	3.7±5.1a	3.8±7.5a	1.4±1.3a	1.0±1.6a	0.2±0.3b	0.5±0.6a
Water Concentrations						
E. coli MPN/100mL	447±762c	16514±48642a	732±930b	206±391b	3324 ±4878a	1227±1812a
TSS -----g L^{-1}------	0.2756±0.5923a	2.0099±5.6008a	0.0704±0.1405b	0.0094±0.0160b	0.1405±0.3239a	0.0074± 0.0101b
pH	7.85±0.38a	7.80±0.37a	7.85±0.38a	7.44±0.35b	7.66±0.22a	7.66±0.31a
Conductivity ----µS/cm----	351.9±150.0b	498.3±477.7a	387.9±169.4ab	221.7±114.0b	354.0±147.7a	312.2±152.7a
Water Loading						
E. coli $1*10^9$ MPN/day	3.92±11.22b	31.49±66.27a	2.18±5.10ab	0.21±0.45a	0.58±1.17a	0.51±0.88a
TSS -- 10^3 g day^{-1}--	294.4±1051.7a	2712.0±6941.3a	11.0±26.3a	0.87±2.1a	2.5±8.3a	0.34±0.48a

Water E. coli Concentrations

E. coli concentrations were highly variable in the roadside ditch water and ranged from less than 1 MPN/100mL to greater than 241,960 MPN/100mL with an overall mean of 4,616.1 MPN/100mL and standard deviation of 24,033.7 MPN/100mL. The data was highly skewed, with two sampling days having a detection limits greater than 24,916 MPN/100mL (Figure 3). The variability was observed at two time scales, within a storm and among seasons. During a storm, *E. coli* concentration peaks corresponded with or occurred immediately following the peak in water flow and total suspended solids concentrations. Low concentrations during the spring and fall often made peaks almost undetectable. First flush and flashy patterns were also exhibited. See Appendix B for examples.

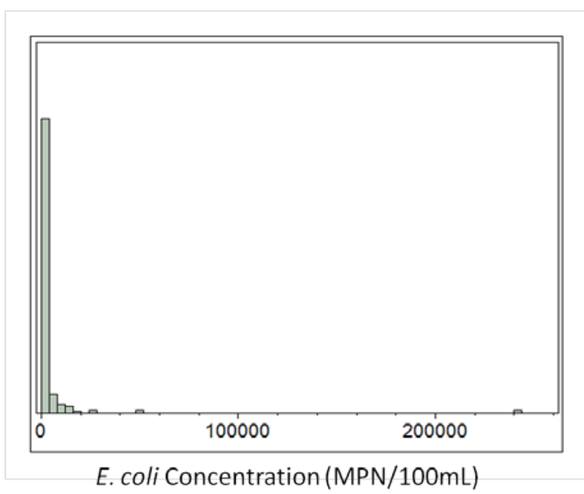
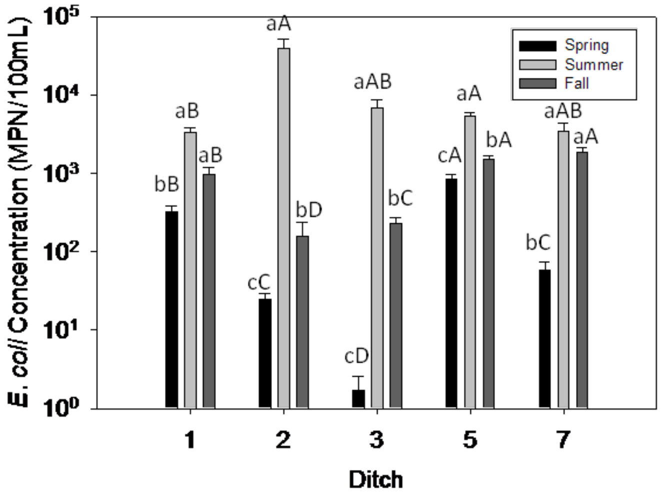


Figure 3. Histogram of the *E. coli* concentrations in roadside ditch water.

The seasonal pattern of *E. coli* concentrations in the roadside ditches adjacent to agriculture was strong, while the pattern in forest roadside ditches was inconsistent. Combined across land uses, summer had significantly higher concentrations of *E. coli* than fall, which had significantly higher concentrations than spring. For the agricultural sites, the concentrations were highest in the summer, declined during the fall and were lowest during the spring (Table 5). This pattern was observed in each agricultural roadside ditch (Figure 4). For the forest ditches, fall and summer were both significantly higher than spring. Though this pattern was observed within each forest roadside ditch (Figure 4), it should be noted that these two ditches functioned differently. Ditch 1 had similar seasonal trends to that of the agricultural sites; it peaked in the summer, declined in the fall, and was lowest during the spring. Ditch 7, on the other hand, did not flow during the first summer and only produced 2 samples during the second summer. The water *E. coli* concentrations in this ditch were relatively low throughout but peaked in fall, declined during the spring and increased again during the summer (See Appendix C for detailed figures of ditch water *E. coli* concentrations).



† Ditches 2, 3 and 5 are adjacent to agricultural fields. Ditches 1 and 7 are adjacent to predominately forest land.
‡Within a ditch, seasons that share similar lower case letters are not significantly different from one another at a 0.05 level according to the analysis of variance test. Within a season, ditches that share a similar capital letter are not significantly different from one another at a 0.05 level according to the analysis of variance test. Throughout this table, the largest means are always denoted by A and get smaller with each consecutive letter. Error bars represent the standard error.

Figure 4. Significant differences among log *E. coli* concentrations among ditches by season.

The presence and timing of manure spreading is likely the driver of the stronger seasonal trend in agricultural *E. coli* concentrations. They peaked immediately after manure spreading and then declined during the fall and spring. The *E. coli* concentration decline with an increasing number of days since manure spreading is described by Figure 5. It is therefore likely that runoff from fields to roadside ditches will exceed the least stringent EPA water regulation of 575 MPN/100mL for 115 days after spreading. This is likely a conservative estimate, as the two concentrations that occurred on the same day as spreading were at the detection limit. To compare the decline in *E. coli* from the agricultural sites to the forest sites, a similar figure was produced except that the average number of days since the average manure spreading date was used. Concentrations of *E. coli* also decreased with time in the forested sites, but the decline was less steep. Ditch 2 provided a case study on the impact of manure amendment on *E. coli* concentrations, as samples were collected before spreading, on the same day as spreading and following spreading (Figure 6). On May 16, 2009, before spreading occurred, the *E. coli* concentration for the single sample collected was 866.4 MPN/100mL. On May 29, 2009 and June 12, 2009, five of six bottles reached the detection limit of 48,392 MPN/100mL and 241,960 MPN/100mL, respectively. Concentrations decreased by at least twenty-fold after only ten days had elapsed and the soil was tilled. The exact magnitude of the decline in unknown, since the samples were at the detection limit.

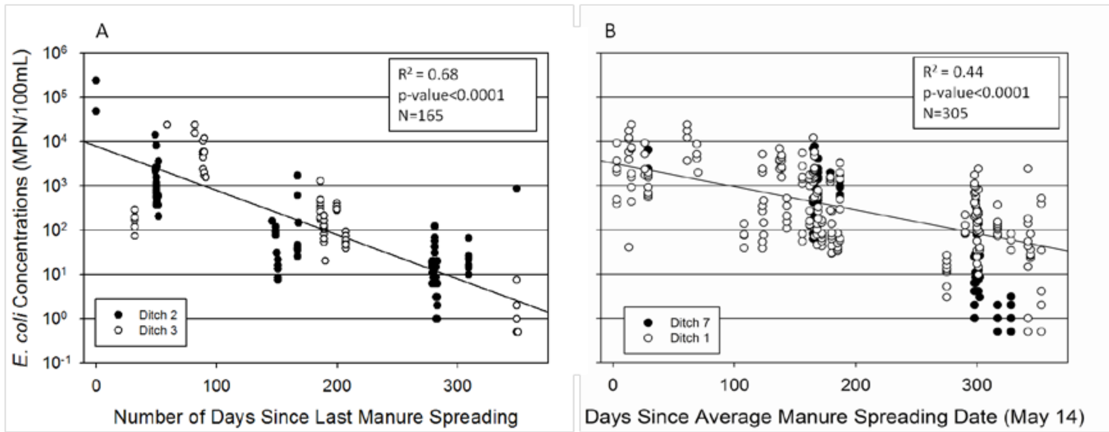


Figure 5. *E. coli* concentrations in roadside ditch water through time based on manure spreading dates. A) The *E. coli* concentrations in agricultural roadside ditch water decrease with an increasing number of days since the last manure spreading event (Log *E. coli* concentration = 3.91 – [0.010 x # of days since last spreading]). Only data from Ditches 2 and 3 were included because these were the only two ditches with known spreading dates. The highest two points are the detection limit for those dates. B) *E. coli* concentrations also decline though time in the forested sites (Log *E. coli* concentration = 3.75 - 0.007 x # of days since average manure spreading date).

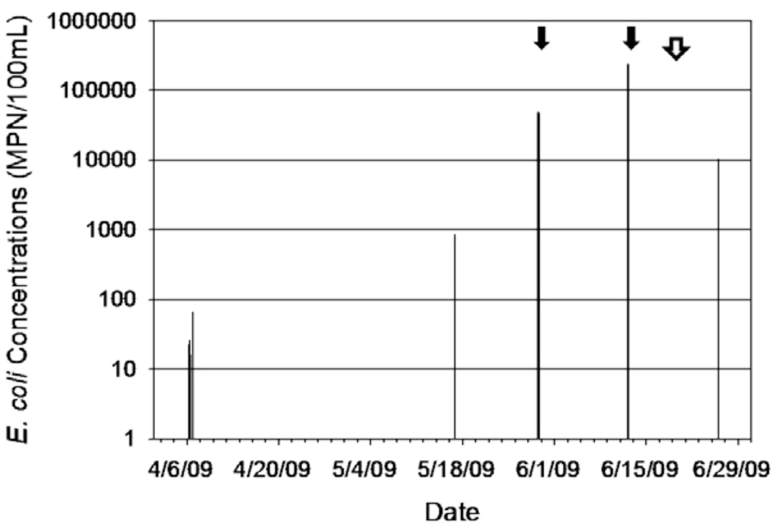


Figure 6. *E. coli* concentrations in roadside ditch water prior to manure spreading for the year, on days where spreading occurred (black arrow) and after soil was tilled (white arrow) for Ditch 2.

When analyzing differences between land uses, geometric mean concentrations of *E. coli* were significantly higher in roadside ditches with agricultural contributing basins than in roadside ditches with forest contributing basins (Table 4). Agriculture was significantly greater than forest during summer. Interestingly, agriculture was significantly less than forest during fall and there was no significant difference between land uses during the spring.

E. coli concentrations were compared to discharge to determine if increased flow mobilized more organisms or if the concentrations were diluted with increased water levels. Overall, *E. coli* concentrations increased with increased discharge (Log *E. coli* concentrations (MPN/100mL) =3.57+0.38 x log discharge (m^3/s), R^2=0.069, $p<0.0001$). This relationship was less consistent when analyzed at the ditch and seasonal levels and was either positive or not significant (Table 6). A positive relationship between *E. coli* and discharge suggests that the rate of increase in the number of *E. coli* mobilized was greater than the increase in flow. On the other hand, no significant relationship between flow and *E. coli* concentrations suggests that flow and the *E. coli* load transported increased at the same rate.

Log *E. coli* concentrations were compared to log total suspended solids concentrations to determine if the *E. coli* was being carried attached to sediment and/or by the same processes as sediment. There was a significant relationship between *E. coli* and total suspended solids (Log *E. coli* concentrations (MPN/100ml) = 3.36+0.47xlog total suspended solid concentration ($g\ L^{-1}$), $R^2 = 0.20$, $p<0.0001$). Due to the high variability of *E. coli* concentration among ditches and seasons, the regression was also analyzed for each ditch and each season. Ditches 1 and 2 showed a consistent positive relationship, while the other sampling sites were variable across the seasons (Table 7).

Table 6. Regression analysis of the relationship between *E. coli* concentrations (log MPN/100mL) and discharge (log m^3s^{-1}) and associated R^2 values.

	Overall†	Spring	Summer	Fall
Agriculture	↑ (0.1056)	↑ (0.7543)	NS (0.0537)	↑ (0.5799)
Ditch 2	NS (0.0076)	↑ (0.6957)	↑ (0.3637)	NS (0.2282)
Ditch 3	NS (0.0856)	NS (0.3249)	NS (0.0326)	NS (0.0007)
Ditch 5	↑ (0.2003)	↑ (0.5808)	↑ (0.2556)	NS (0.1815)
Forest	NS (0.0074)	NS (0.0768)	NS (0.1058)	↑ (0.1000)
Ditch 1	NS (0.0079)	NS (0.1681)	↑ (0.1660)	NS (0.1160)
Ditch 7	NS (0.0257)	↑ (0.6669)	NS (0.3025)	NS (0.0196)

† NS denotes no significant relationship at a 0.001 level. ↑ denotes positive relationship at a 0.001 level. ↓ denotes an inverse relationship at a 0.001 level.

Table 7. Regression analysis of the relationship between *E. coli* concentrations (log MPN/100mL) and total suspended solids concentrations (log g L^{-1}) and associated R^2 values.

	Overall†	Spring	Summer	Fall
Agriculture	↑ (0.1604)	↑ (0.4987)	↑ (0.2527)	↑ (0.1651)
Ditch 2	NS (0.0048)	↑ (0.4348)	↑ (0.7269)	↑ (0.4698)
Ditch 3	NS (0.0032)	NS (0.4993)	NS (0.4936)	NS (0.0000)
Ditch 5	↑ (0.4044)	↑ (0.5019)	↑ (0.6723)	NS (0.0059)
Forest	↑ (0.2458)	↑ (0.2860)	↑ (0.2807)	↑ (0.1457)
Ditch 1	↑ (0.2848)	↑ (0.5176)	↑ (0.2720)	↑ (0.1654)
Ditch 7	↑ (0.3650)	↑ (0.3096)	NS (1) ‡	NS (0.1198)

† NS denotes no significant relationship at a 0.001 level. ↑ denotes positive relationship at a 0.001 level. ↓ denotes an inverse relationship at a 0.001 level.
‡ Only two samples were collected during this season for Ditch 7

Water E. coli Loading

Overall, summer had significantly higher *E. coli* loads than spring and agricultural roadside ditches had higher loads than forest roadside ditches (Table 4). For the agricultural sites, summer was significantly higher than spring, while there was no significant difference for the forest among the seasons (Table 5). Agricultural roadside ditches were only significantly greater than forest sites for summer, with no difference between land uses for spring and fall. From analysis on the individual ditch level, Ditch 5 is the largest contributor of *E. coli.* This ditch was significantly greater than at least one other ditch for all seasons, while no other ditch was consistently low or consistently high.

According to our model for scaling up to the watershed scale, the roadside ditch networks did carry large enough loads to cause the streams to have a higher concentration of *E. coli* than the EPA recommendation of 575 MPN/100mL. Estimated roadside ditch densities ranged from 0.001875 to 0.002425 m/m^2 (Table 8). The average daily *E. coli* load per meter of roadside ditch for agriculture and forest was 60,071,044 and 1,902,158 MPN, respectively. The maximum loading was on May 16, 2009 and predicted stream concentrations reached as high as 660,000 MPN/100mL. When the loading and stream discharge was combined, the Watershed 1, 2, 3 and 4 were above the standard for 24, 20, 28 and 48% of the days sampled, respectively (Table 8). These results must be viewed with caution for a few reasons. First, the average water flow in the streams for each season was used, while the ditch contributions occur during high flow runoff events. Second, it is unknown whether the ditch density used is applicable to each watershed. Regardless, this method shows that ditches have the potential to carry loads in the order of magnitude required to cause streams to be out of compliance.

Table 8. Input parameters for the model to determine the total cumulative loading from the roadside ditch network.

Watershed	Agricultural Land Area (m^2)	Non-agricultural Land Area (m^2)	Road Length (m)	Road Density (m/m^2)	Roadside Ditch Density (m/m^2)	% of sampling days exceeding 575 MPN/100mL
1	35154900	44214300	117953	0.001486	0.002425	24
2	123796800	356597100	607093	0.001264	0.002063	20
3	164408400	65448000	321729	0.001399	0.002284	28
4	31230900	9630900	46961	0.001149	0.001875	48

Total Suspended Solids Concentrations

Similar to *E. coli* concentrations, there was a high degree of variability in total suspended solids concentrations, which ranged from 0.0000 to 52.22 g L^{-1}, with an overall mean of 0.51 g L^{-1} and a standard deviation of 2.74 g L^{-1}. Within a storm, the TSS tended to peak at the same time as flow, but did sometimes peak before the peak flow and sometimes did not peak at all.

Agricultural roadside ditches had a significantly higher concentration of total suspended solids than forest roadside ditches overall and for each season (Table 4). As with the *E. coli,* there is some variability of rankings within the land uses and among the individual ditches. Ditch 5 was always significantly higher than at least one other ditch for TSS, while Ditch 3 was always significantly lower than at least one other ditch, though both were agricultural ditches.

Unlike the *E. coli* concentrations, there was not a consistent seasonal pattern for total suspended solids concentrations. When all the samples were combined, summer had significantly higher concentrations than spring and fall. At the land use level, spring and summer were significantly greater than fall for agricultural ditches, while spring and fall were significantly greater than summer for the forest sites (Table

5). The overall trends for each land use did not apply to the individual ditch. Spring and summer were greater than fall for Ditch 5, an agricultural ditch, and Ditch 1, a forest ditch. In Ditch 3, an agricultural ditch, there was no significant difference in concentrations among the three seasons, though 13 of the 20 highest concentrations were in the fall. Spring had significantly higher concentrations of TSS than summer, while there was no significant difference in concentrations among fall and the other seasons for Ditch 2, another agricultural ditch. Ditch 7, a forest site, had significantly higher concentrations in the summer than fall, which was significantly higher than the spring.

Total Suspended Solids Loading

Overall, there was no difference in TSS loads among the seasons. This held true for loads at the land use level (Table 5). At the ditch level, only Ditch 2 had a seasonal pattern with spring being significantly higher than summer. Overall and within each season, agricultural roadside ditches had higher TSS loadings than forest roadside ditches (Table 4). Again, Ditch 5 had the largest TSS contributions than any of the other ditches.

Water Chemical Properties

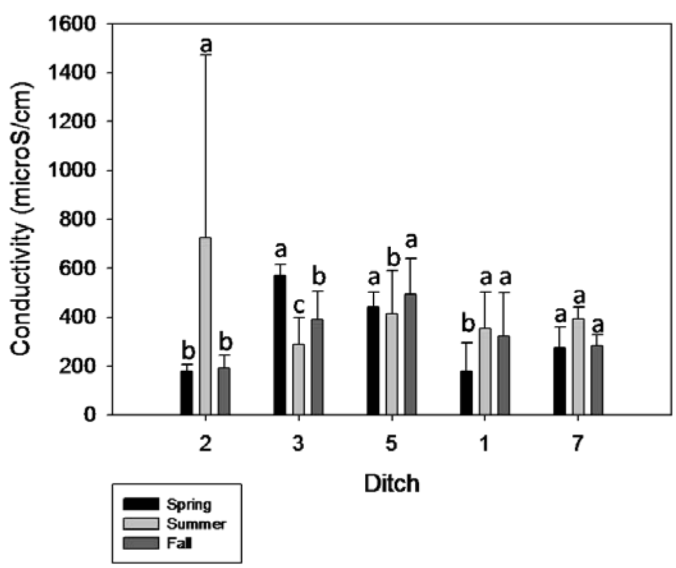
<u>pH</u>

The pH of water samples ranged from 6.66 to 8.67, with a mean of 7.71 and standard deviation of 0.37. When all samples were combined, summer (mean = 7.75) and fall (mean=7.74) had significantly higher pH values than spring (mean=7.63; ANOVA, p=0.0010). Two different trends emerged within each land use though. There was no significant difference among the seasons for the agricultural ditches, but summer and fall had significantly higher mean pHs than spring for the forest sites

(Table 5). The agricultural roadside ditches had a significantly higher mean pH than the forest roadside ditches overall and by season.

<u>Conductivity</u>

The conductivity ranged from 68.0 to 2370 µS/cm, with an overall mean of 358 µS/cm and standard deviation of 270 µS/cm. When all the samples were combined, summer (mean=359 µS/cm) had a significantly higher mean conductivity than fall (mean=310 µS/cm), which had a significantly higher conductivity than spring (mean= 245 µS/cm; ANOVA, $p<0.0001$). Summer had significantly higher conductivities than spring in the agricultural sites, while summer and fall had significantly higher conductivities than spring in the forest sites (Table 5). This pattern was not observed at the ditch level though (Figure 7).



†Seasons within the same ditch with the same letter are not statistically significant at the 0.05 level (a>b>c). Error bars represent the standard deviation.

‡Ditches 2, 3 and 5 are agricultural, while Ditches 1 and 7 are forest.

Figure 7. Mean conductivities by season for each ditch.

The agricultural roadside ditch water samples had significantly higher conductivity levels than forested sites overall and for each season (Table 4). The largest conductivities were not during the snowmelt event, as expected, but were in Ditch 2 on the days when sample collection occurred on the same day as spreading, with values ranging from 1794 – 2370 µS/cm. The next largest value, 1266 µS/cm, was from Ditch 1 in early November. It is interesting to note that for fall and spring, Ditch 2 was significantly lower than at least one other ditch (Fall: 3,5>1,7>2; Spring: 3,5>7>2,1), while it had significantly higher conductivities for summer than Ditches 1 and 3.

Sediment E. coli

A total of 182 sediment samples were collected from the ditch bottoms on 7 different dates between storm events. The *E. coli* levels ranged from less than 1 to greater than 2419.6 MPN with a mean of 331.8 and a standard deviation of 679.8.

Overall, the agricultural roadside ditch sediment had significantly higher concentrations of *E. coli* than forest sites (Table 4). To determine if trends of *E. coli* decline occurred in the sediment, the *E. coli* levels were plotted through time (Julian Date after July 1). For 2 of the 4 agricultural ditches (Ditches 3 and 5), the sediment *E. coli* exhibited a significant (α=0.01) linear decline from July to November 2008 and the third exhibited a decline that was not statistically significant. Ditch 6 did not have any water flow and the manure spreading schedule was unknown; it exhibited a uniquely significant increase in concentrations with days from July 1. On the other hand, the forest sites did not show any trend in sediment *E. coli* (Table 8).

Table 9. Regression analysis of the relationship between *E. coli* concentrations and time. Date represents the Julian Date starting July 1 for a leap year.

Ditch	Equation	R^2	p-value
Agricultural			
2	*E. coli* (MPN) = 3021 - 8.58xJulian Date	0.11	0.0948
3	*E. coli* (MPN) = 1203 - 4.11xJulian Date	0.34	0.0018
5	*E. coli* (MPN) = 2889- 9.92xJulian Date	0.39	0.0006
6	*E. coli* (MPN) = -1292+ 5.70xJulian Date	0.18	0.0319
Forest			
1	*E. coli* (MPN) = -26 + 0.19xJulian Date	0.053	0.2575
4	*E. coli* (MPN) = -21 + 0.58xJulian Date	0.002	0.8225
7	*E. coli* (MPN) = 957- 1.21xJulian Date	0.002	0.8166

Sediment moisture content was variable and ranged from 2.4 to 139.3%. The mean moisture content was 33.4% with a standard deviation of 23.3%. The agricultural roadside ditch sediment (mean: 41.5%, standard deviation: 24.8%, range: 3.2 to 139.3%) had a significantly higher moisture content than forest sites (mean: 22.6%, standard deviation: 15.7%, range: 2.4 to 65.7%) (R^2=0.16, $p<0.0001$). Sediment moisture content was not a contributing factor to *E. coli* levels, as there was no correlation between *E. coli* concentrations and sediment moisture content by individual ditch, by adjacent land use or an aggregation of the samples as a whole.

DISCUSSION

Roadside ditches are a conduit for the fecal indicator organism *E. coli* and potentially for pathogens from the landscape to streams and drinking water supplies. High concentrations of *E. coli* were found in ditches adjacent to manure amended agricultural fields. Peak concentrations were associated with manure spreading and declined through time but were still detectable months after spreading. *E. coli* concentrations were also high in roadside ditches adjacent to forest lands, with possible sources including septic systems, pets, wildlife and livestock. The ditches monitored in this study are only a small portion of the road drainage network in rural landscapes. When scaled up to the entire watershed, it was predicted that roadside ditches carry loads of *E. coli* large enough to degrade stream water quality to beyond compliance. Sediments in roadside ditch bottoms act as a reservoir for *E. coli* between storm events with the potential for resuspension. Roadside ditches rapidly convey water through the watershed and reduce the ability of natural processes, such as desiccation and infiltration, to decrease viable pathogen loads downstream. Roadside ditch management is therefore a critical target in the prevention of pathogen transport and waterborne disease.

This study was the first to document high concentrations of fecal indicator organisms in roadside ditch water. The concentrations within the roadside ditches were well above the New York State Department of Environmental Conservation (NYS DEC) regulations and the US EPA recommendations. For New York State, fecal coliform monthly geometric mean should not exceed 200 FC/100 mL for all classes of freshwater (New York State Department of Environmental Conservation, 2008b). The US EPA recommendations state that the geometric mean should not exceed 126 *E. coli*/100 mL overall and for infrequently used full body recreation (the least stringent recommendation) should not exceed 575 *E. coli/* 100 mL for a single sample (United State Environmental Protection Agency, 2004). Of the 629 water samples analyzed, 60% exceeded the NYS DEC regulations, and 48% exceeded the US EPA recommendation of 575 MPN/100mL. Roadside ditches are carrying water with high concentrations of fecal indicator organisms to streams and therefore drinking water supply systems.

Though concentration is an important indicator, bacterial loading elucidates the contribution of each roadside ditch, when normalized for differences in flow. A ditch with high concentrations but a low flow may contribute fewer organisms to a stream than one with a lower concentration and higher flows. The largest *E. coli* load was not from Ditch 2 on May 29, 2009 or June 12, 2009, when concentrations were greater than the detection limits of 48,392 MPN/100mL and 241,960 MPN/100mL, respectively. Instead, daily loads were greater in Ditch 5 on 5 dates, due to the combined effects of high concentrations with high flow. Daily loading from agricultural roadside ditches ranged from 0 to 2.75×10^{11} MPN/day with discharges ranging from 0.32 to 4550 $m^3 day^{-1}$, while loading ranged from 0 to 4.32×10^9 MPN/day in the forest sites with discharges ranging from 0.02 to 726 $m^3 day^{-1}$. The daily loading from individual ditches during storm events were similar in orders of magnitude to those reported in similar studies on streams and tributaries (Table 10). In addition, our estimates on the cumulative impact of roadside ditches showed that ditch network loading was large enough to be detectable in the streams and produce stream concentrations above the NYS DEC and EPA recommendations. Though the impact of an individual ditch is likely to be diluted out, the ditch network has the potential to carry large enough loads to degrade stream water quality.

Table 10. **Summary of studies with *E. coli* loadings.**

Study site description	**Fecal Indicator Organism**	**Time period**	**Daily load**
This study	*E. coli*	Daily	0 to 2.75×10^{11} MPN/day (3.67×10^{-6} to 0.053 $m^3 s^{-1}$ (0.32 to 4550 $m^3 day^{-1}$)
Eagle Creek Watershed Mixed land use Indianapolis, Indiana (Vidon, et al., 2008)	*E. coli*	Daily	• Baseflow: 3.61×10^{10} to 48.25×10^{10} MPN/day (0.05 to 0.12 $m^3 s^{-1}$) • High flow: 170.77×10^{10} to 742.32×10^{10} MPN/day (0.90 to 2.00 $m^3 s^{-1}$) • Geometric means
Graywood Gully Predominately agriculture Conesus Lake, NY (Robert D. Simon & Joseph C. Makarewicz, 2009)	*E. coli*	Event (average 84.3 hours)	10^{10} CFU to 10^{13} CFU per event (Up to 12,293 m^3 day^{-1})
Tributaries to drinking water reservoirs in Germany (Kistemann, et al., 2002)	*E. coli*	Average 12-hour event	• Pristine with deer: 2.28×10^{10} CFU (12,000 m^3) • Predominately grazed: 7.17×10^{11} CFU (55200 m^3) • With wastewater treatment plant: 6.16×10^{12} CFU (44900 m^3)
Stock Creek Mixed land use Tennessee (Gentry, et al., 2006)	*E. coli*	Daily	• Headwaters: 0.67×10^{10} CFU day^{-1} (0.6 m^3 s^{-1}) • Outlet: 14.58×10^{10} CFU day^{-1} (4.84 m^3 s^{-1}) • Geometric means
Thomas Creek Nova Scotia Agriculture and residential (Jamieson, et al., 2003)	Fecal coliform	Between May and December 8 month time period	• Dairy: 4.9×10^{13} (0.16 $m^3 s^{-1}$ – average daily flow) • Residential: 3.2×10^{13} (0.22 m^3 s^{-1} – average daily flow)

Relevant factors influencing the concentrations and loading of *E. coli* examined in this study included adjacent land use, season, discharge and total suspended solids concentrations. Land management contributed to elevated *E. coli* levels within roadside ditches. Overall, ditches with manure amendment in the contributing area had significantly higher concentrations and significantly higher loadings. High concentrations of *E. coli* were expected in agricultural roadside ditches due to the extensive research showing high levels of fecal indicator organisms in streams from manure amendment and grazing (Jamieson, et al., 2003; Niemi & Niemi, 1991; Patni, et al., 1985; R. D. Simon & J. C. Makarewicz, 2009). Roadside ditches are therefore a conduit of fecal indicator organisms from the field to the stream. Though significantly lower, high concentrations were still observed in the forest sites. Likely alternative sources for *E. coli* in these ditches may include leaking septic or sewer systems, wildlife, pets or naturalized *E. coli* populations. A study conducted by Somarelli, Makarewicz et al (2007) completed source tracking of *E. coli* in Conesus Lake, Finger Lakes, NY. They found geese were the dominant source of *E. coli*, followed by cows, deer and finally humans, even though three of the four sub-basins had livestock in them. Ishii, Ksoll et al. (2006) found *E. coli* in soils near Lake Superior that were genetically similar in the same site but genetically different than water inputs and known wildlife strains. These identified strains persisted through the freeze-thaw cycles of winter and grew during the summer months, suggesting that the strains had become "naturalized" and therefore potential sources of *E. coli* in runoff. In addition, Ditch 7 was located on the edge of a state park, where many people walked their dogs and feces were observed in the ditch itself. Ditch 1 had a small home with a tile drain running down into the roadside ditch and was within 2 km of a large dairy operation. Niemi and Niemi (1991) found that pristine areas in close proximity to agriculture tended to have elevated levels of fecal indicator organisms. It is therefore likely that human, pet, wildlife and even livestock were also contributing to the *E. coli* concentrations in our predominately forested sites. Adjacent land use, specifically manure amended agriculture, was a contributing factor to the loading of fecal indicator organisms to the roadside ditch network.

Indicator organism concentration and loading exhibited a seasonal pattern. There were higher concentrations of *E. coli* during summer when compared to spring for both agricultural and forest sites and when compared to fall in the agricultural sites. On the other hand, *E. coli* loading was greater in summer only for agricultural sites. Within the agricultural sites, manure spreading is likely the cause of the elevated levels of *E. coli* during summer, because we defined our summer season as the time period after spreading. Studies have also found higher levels of fecal indicator organisms in summer for the Hoosic River, MA (Traister & Anisfeld, 2006), Cornwallis River sub-catchment in Nova Scotia (Jamieson, et al., 2003) and streams contributing to Conesus Lake(R. D. Simon & J. C. Makarewicz, 2009). These peaks were also found in sites that were not impacted by livestock (Jamieson, et al., 2003; R. D. Simon & J. C. Makarewicz, 2009). During the summer, possible explanations for increased concentration in forest sites could be that wildlife may be more active, more people may be walking their dogs and water volumes flowing through the ditches were reduced. Though peak *E. coli* concentrations were found during the summer, viable *E. coli* was also found during the fall and spring. Source tracking was beyond the scope of this study, so the exact sources of *E. coli* are unknown. Viable organisms in the late fall and spring may be from naturalized populations of *E. coli* and from wildlife. It is also possible that viable *E. coli* are surviving in the soil for months. A review by Rogers and Haines (2005) documented the range of longest reported survival times for pathogens in soil. The range in survival of *E. coli* O157:H7 was from greater than 300 days for temperatures of -20 to -4^{o}C to as low as 32 days for manure amended soils at 0 to 22^{o}C. Soils with dairy manure slurry amendment had survival of 64 days. However, Fenlon, Odgen et al. (2000) reported survival of *E. coli* for more than 20 weeks in soils exposed to ambient temperatures in Scotland. In addition, Ogden, Fenlon et al. (2001) reported that *E. coli* in soil columns declined in a biphasic way, with a susceptible population having a half life of 3 to 4 days and a resistant population with a half life of 18 to 24 days. This suggests that although initial die off is fast, there may be a resistant population that survives longer in the soil and is less susceptible to temperature and moisture controls.

The impact of water flow and total suspended solid concentrations on *E. coli* concentrations was variable. Overall, there was a positive relationship between discharge and *E. coli* concentrations, but this relationship was not true for each ditch in each season. Vidon, Tedesco et al. (2008) had similar findings in their study on stream *E. coli* concentrations and loadings in Indianapolis, Indiana. They found an overall correlation between average daily discharge and *E. coli* concentrations, but the correlation was not consistent across sites or flow conditions (Vidon, et al., 2008). Elevated flows in different seasons may be marked by different processes. In central New York, runoff events in summer are from short, intense storms. In the spring and fall, when less evapotranspiration is occurring and the soil is saturated, less precipitation is required to produce similar flows. This hypothesis was supported by Wilkes, Edge et al. who found *E. coli* was better correlated with cumulative rainfall than with discharge (2009). In addition, Simon and Makarewicz (2009) compared *E. coli* and total suspended sediment concentrations during events, defined as a stream level rise $\geq$2.54cm/30min, and non-events with similar discharge values. *E. coli* and total suspended sediment concentrations were significantly higher during events than non-events. Therefore, the relationship between flow and *E. coli* concentrations may change by season and depend on hydrologic processes.

Many studies have documented a positive correlation between fecal indicator organism concentrations and total suspended solids concentrations. Like flow, there was an overall positive relationship between total suspended solids and *E. coli* concentrations in the roadside ditch water. Positive relationships were found during at least one season in Ditches 1, 2, 5 and 7. This association has two possible explanations. First, many studies in the past have documented that fecal coliform often are transported attached to sediment particles, though the exact partition has shown some variability (Characklis, et al., 2005; R.C. Jamieson, et al., 2005; Muirhead, et al., 2006a; Oliver, et al., 2007). In addition, the fraction of *E. coli* attached to settleable particles remained constant over the course of the storm events, even with changing *E. coli* concentrations throughout the storm (Krometis, et al., 2007). This suggests that there should be a positive relationship between *E. coli* and total suspended solids. This relationship may break down during seasons when fewer viable *E. coli* are available for transport. In addition to be transported attached to sediment, *E. coli* may be eroded and transported by the same mechanisms as sediment, which would also lead to a correlation between concentrations. A lack of correlation was found in many ditches and may be due to different processes acting on sediment and sediment associated *E. coli* as compared to free floating *E. coli.* It may also be due to a reduced number of viable *E. coli* available to be transported during certain seasons.

Roadside ditches are not only a conduit of fecal indicator organisms, but they also act as a refuge for survival. Viable organisms were found in the sediment of roadside ditches in this study between storm events at high concentrations. The concentrations were highly variable with the maximum range for a single date within a single ditch of over 600,000 MPN/100mL sediment. Some of the variability may be explained by the effects of microclimate within the roadside ditches. Extreme temperatures, extreme pH, moisture, nutrient supply and solar radiation are the most important factors controlling the survival of enteric bacteria in the soil and water environment (Crane & Moore, 1986). This study did determine that sediment moisture content was not a determinant of *E. coli* concentrations, as there was no relationship between moisture content and *E. coli* levels. It was beyond the scope of this study to explicitly examine other microclimatic factors. However, it is unlikely that solar radiation was a driving factor. All ditches were at least 0.28 m deep and all had some vegetation growing along the ditch bottom. We predict that the ditch bottom would remain shaded for the majority of the day. Temperature may be an important difference in sediment *E. coli* levels. Although at the micro-scale, the ditches all had shading, those adjacent to the forest had the additional canopy shading, while those next to agricultural fields were exposed to the sun. A study by Van Donsel, Geldreich et al. (1967) found fecal coliform survived twice as long in soil on a forested hillslope when compared to a flat lawn with sparse vegetation. The interaction of all these factors creates highly variable conditions and therefore variable concentrations of *E. coli* within the ditch bottom sediment.

Other studies have also documented indicator organisms in stream and river bottom sediments. Jamieson and Gordon et al. (2003) found concentrations of fecal coliform in a stream segment below a dairy to be as high as 58,800 MPN/g, while the water concentrations did not exceed 10,000 MPN/100 mL. In the Buffalo River, fecal coliform concentrations in the river bottom sediment were between 10^4 to 10^5 FC per gram during the summer and declined during the winter, while the geometric mean in the water samples were between 2.2 and 34 FC/mL for summer and between 1.6 and

34 FC/mL for winter. In both cases, the concentrations of fecal indicators were much greater in the sediment than in the water column. In this study, the concentrations of *E. coli* in roadside ditch sediments were also higher than the concentrations of *E. coli* in the water the majority of the time. Roadside ditch sediment *E. coli* was significantly higher in agricultural sites when compared to forest sites, but was still present in the forest sites. Other studies have found fecal coliform in sediments that were not impacted by manure amendment and likely had contributions from wildlife (Entry, Hubbard, Thies, & Fuhrmann, 2000). The roadside ditch sediment adjacent to agricultural fields showed a linear decline through time, while there was no trend for the roadside ditch sediment adjacent to forest lands. This may be due to the fact that manure spreading only occurs over a short period of time and would contribute a finite supply of bacteria to the ditch sediment. The sources in the forest settings, including wildlife, people and pets, have more continuous inputs and potentially longer survival times from canopy shading. Regardless of adjacent land use, fecal indicator organisms are found within roadside ditches between storm events at concentrations exceeding those of the water. These organisms may be resuspended during the next storm and contribute to a decline in bacterial water quality in the streams.

Roadside ditch water is transporting large amounts of sediment to the streams. The total suspended solids concentrations ranged from 0.0000 to 52 g L^{-1}. The range of concentrations was similar to that of a previous study on roadside ditches in central New York, which had a maximum concentration of 38.29 g L^{-1}. It should be noted that the peak concentrations remained below 5 g L^{-1} if the percent of exposed bottom was less than 41.7% (Diaz-Robles, 2007). For this study, only one ditch had peak concentrations above 5 g L^{-1}, but all of the ditches had at least some vegetation in the bottoms, so this is not unexpected. The concentrations found in roadside ditches were higher than highway runoff concentrations found in previous studies. Legret and Pagotta (1999) found the mean concentration of total suspended solids for a rural highway in France to be 0.071 with a range of 0.016 to 0.267 g L^{-1}. On a Swedish highway, total suspended solids concentrations were between <0.01 and 1.8 g L^{-1} during the winter and between 0.046 and 0.98 g L^{-1} during the summer (Hallberg & Renman, 2006, 2008). The total suspended solids concentrations were much higher in the roadside ditches, regardless of adjacent land use, than these previous studies. This suggests that the majority of the sediment traveling in the ditch network was not from the road surface itself, but instead was entering the ditches from the surrounding landscape or the ditch itself was a source. This hypothesis is also supported by Wemple and Jones (2003), who found that less than 7% of runoff measured in culverts was from the road surface. Land use did impact total suspended solids concentrations, with agriculture having significantly higher concentrations than forest. Agricultural fields were expected to produce more sediment, as vegetation is temporary and the soil structure is disturbed through tilling. Though total suspended solids concentrations are important for their impact on *E. coli* transport, they also have ecological impacts when transported and deposited in the streams, such as filling gravel beds and reducing light penetration. The New York DEC does not have a quantitative regulation for total suspended solids. The regulation states that concentrations should be below levels that would impair the best usage for that water body (New York State Department of Environmental Conservation, 2008a). It was therefore impossible to determine if/how often roadside ditches had concentrations of solids above a regulation.

Roadside ditches have been documented carrying *E. coli* and sediment in this study and are impacted by adjacent land use. This interaction between land management and roadside ditches is important because ditches are likely increasing the speed at which these indicator organisms and sediment are making it to the streams and drinking water supply systems. Roadside ditches are frequently hydrologically connected to streams and therefore act as first order streams and increase stream channel density (Mills, et al., 2007; Montgomery, 1994; B. Wemple, et al., 1996). These factors increase the speed at which water is traveling through the watershed. Jones and Grant (1996) found an advancement in the initiation of storm flow due to road construction, though not statistically significant, by an average of 10 hours. Because the ditches are moving water quickly, they are bypassing the watersheds natural ability to filter and trap bacteria and sediments. In addition, Brian Buchanan (personal communication, unpublished data) also found more total flow in the streams due to the roadside ditch network. It is therefore possible that land deeper in the watershed, which would not normally be connected directly to the stream network, is now connected to the stream. Therefore, pathogens deeper in the landscape have a conduit to the stream network.

Climate change will likely exacerbate the transport of pollutants in roadside ditches. Incidence of waterborne disease has been linked to extreme precipitation events (Curriero, et al., 2001; Rose, et al., 2001). For the Great Lakes Region, average precipitation and more extreme precipitation events are expected to increase with climate change. A case study on southern Wisconsin found that the wettest days will become more intense, while the case study in the Chicago area found factors contributing to beach closures, including increased magnitude of rainfall, increased water temperature and decreased water stage, are all likely to increase with climate change (Patz, Vavrus, Uejio, & McLellan, 2008). Boxall, Hardy et al. (2009) reviewed the possible impacts of climate change on exposure to pathogens from agriculture in the UK. The impact of climate change on the fate of pathogens in the environment is unknown, as increased temperature, UV and drought will likely lead to death, while warmer, wetter summers and increased flooding may lead to increased survival and growth; expected climate change will likely enhance attributes associated with pathogen death and survival. Though fate is unknown, the increase in precipitation is likely to increase the transport of pathogens (Boxall, et al., 2009). Precipitation is a key driver in pathogen transport and source water contamination. With climate change, targeted management of roadside ditches will become more important, as precipitation intensity will likely increase the number of days and/or amount of flow and the associated pollutants transported via the roadside ditch network.

More research is needed on the efficacy of different management options to improve water quality and reduce the quantity of water in the roadside ditch network. The first target should be land management in the contributing basin. On the farm, Meals and Braun (2006) found that *E. coli* concentrations in runoff from experimental plots in Vermont were reduced when manure was stored for 30 days and when manure was spread 3 days prior rather than on the day of the rainfall event. Our results also documented a decrease in *E. coli* concentrations with increased time since manure spreading. In addition to manure management, the use of vegetated filter strips between the field and the roadside ditches may improve water quality. Lim, Edwards et al. (1998) found that after passage through a 6.1 meter vegetated buffer strip, fecal coliform concentrations went from 1.8×10^6 FC/100mL to undetectable with infiltration as the proposed mechanism. On the other hand, Tate, Atwill et al. (2006) found the efficacy of the filter strip was decreased as the runoff volume increased. Coyne, Gilfillen et al. (1998) determined that removal efficiencies were 75% from a 4.5 meter filter and 91% for a 9.0 meter filter, but concentrations still exceeded water quality recommendations. Though past research has found removal of indicators in filter strips, their applicability to improving water quality to roadside ditches should be further explored.

In addition to improving water quality entering the ditch network, it will be important to reduce the hydrologic connections between the land, the ditches and the streams. Though not explicitly examined in this study, tile drains were observed directly connecting fields and residential areas to the roadside ditch network. Subsurface drainage systems have been shown to change field scale hydrology and also transport fecal indicator organisms (Blann, Anderson, Sands, & Vondracek, 2009; Fenlon, et al., 2000; Oliver, Heathwaite, Haygarth, & Clegg, 2005). Disconnecting tile drains from the roadside ditch network through infiltration basins or constructed wetlands may be a means of preventing pathogen transport. Similarly, constructing or maintaining existing wetlands to receive roadside ditch water or runoff from fields could help reduce both bacterial and sediment loading to streams. Reinoso, Torres et al. (2008) found facultative ponds, surface flow wetlands and subsurface flow wetlands removed 97, 38 and 72% of *E. coli,* respectively, though water concentrations were still above recommended levels. In small wetlands with hydraulic residence times below 2 hours, *E. coli* and sediment were reduced, though the channelized wetland sometimes acted a source of sediment (Knox, Dahgren, Tate, & Atwill, 2008). Future research should focus on ways of managing the land and roadside ditches to improve water quality within the ditches and finding cost effective ways of reducing the hydrologic connection between the land, ditches and stream networks.

CONCLUSION

Roadside ditches are ubiquitous through the landscape and are designed to effectively transport water away from the road. The unintended consequence is that they are also carrying pollutants quickly to the streams. Roadside ditches are carrying significant concentrations and loads of *E. coli* and total suspended solids. They are also acting as a reservoir for *E. coli* which can then be resuspended in the next storm. Adjacent land use affects the pollutant concentrations and loading and may be used as a way to indentify high risk areas. Watershed managers should engage highway managers to achieve the common goal of reducing the impacts of roadside ditches on water quality downstream.

APPENDIX A: Rating Curves

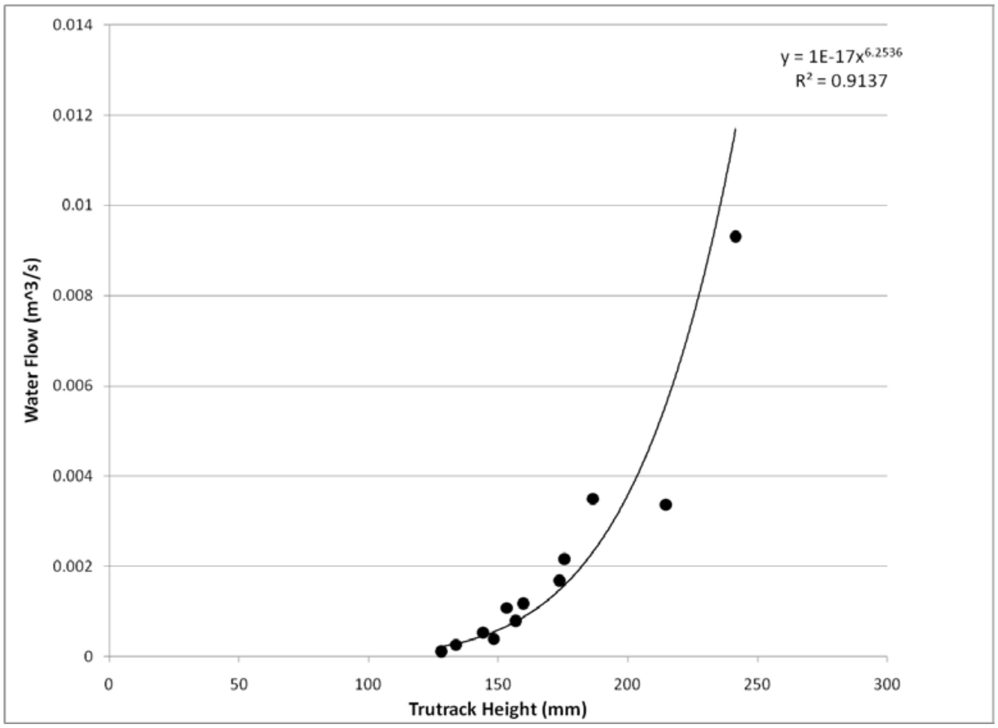


Figure A 1. Ditch 1 rating curve.

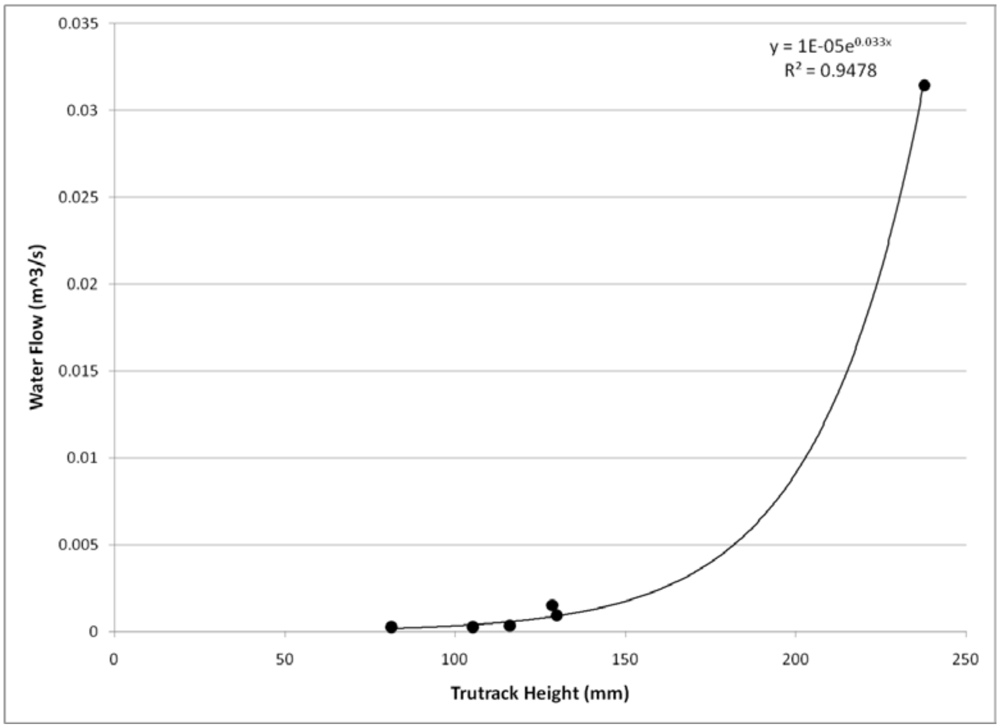


Figure A 2. Ditch 2 rating curve.

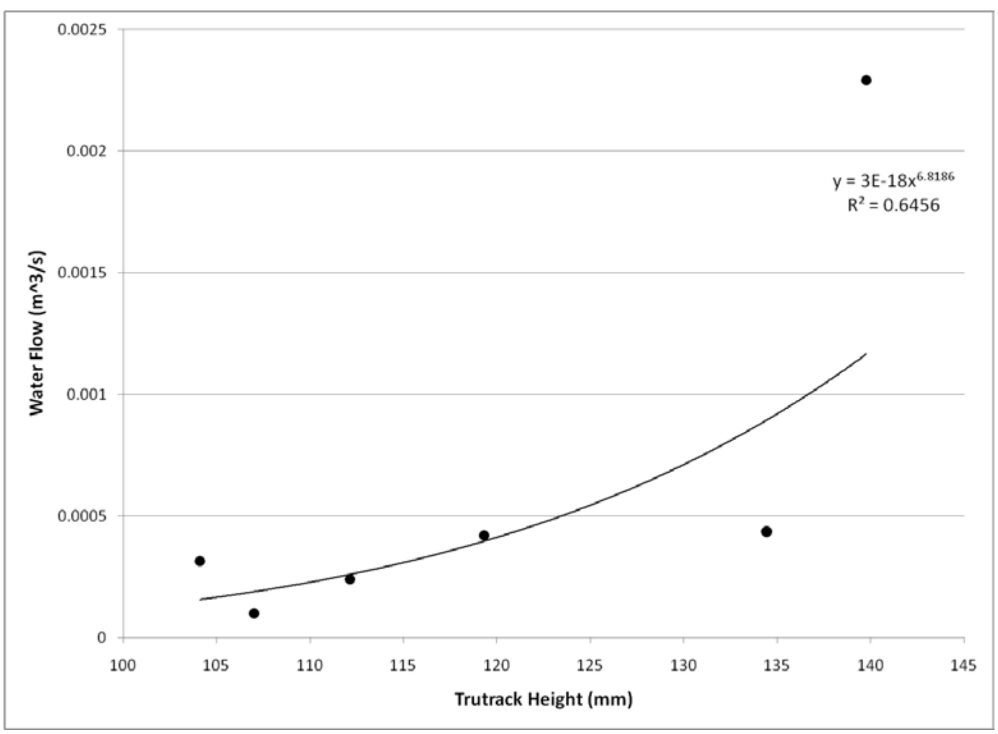


Figure A 3. Ditch 3 rating curve.

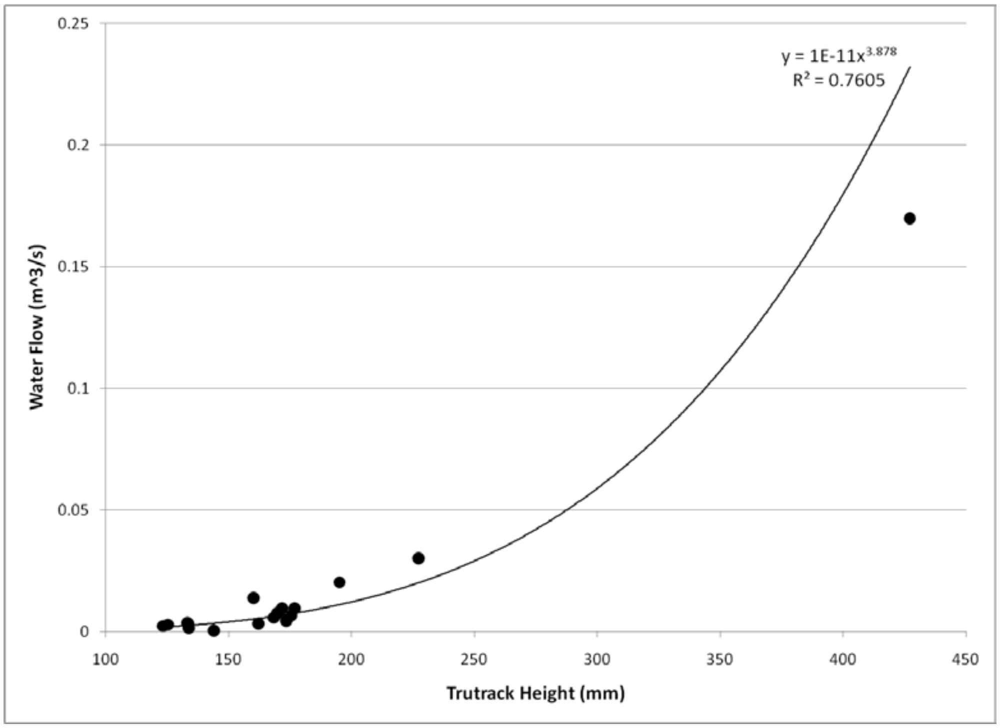


Figure A 4. Ditch 5 rating curve.

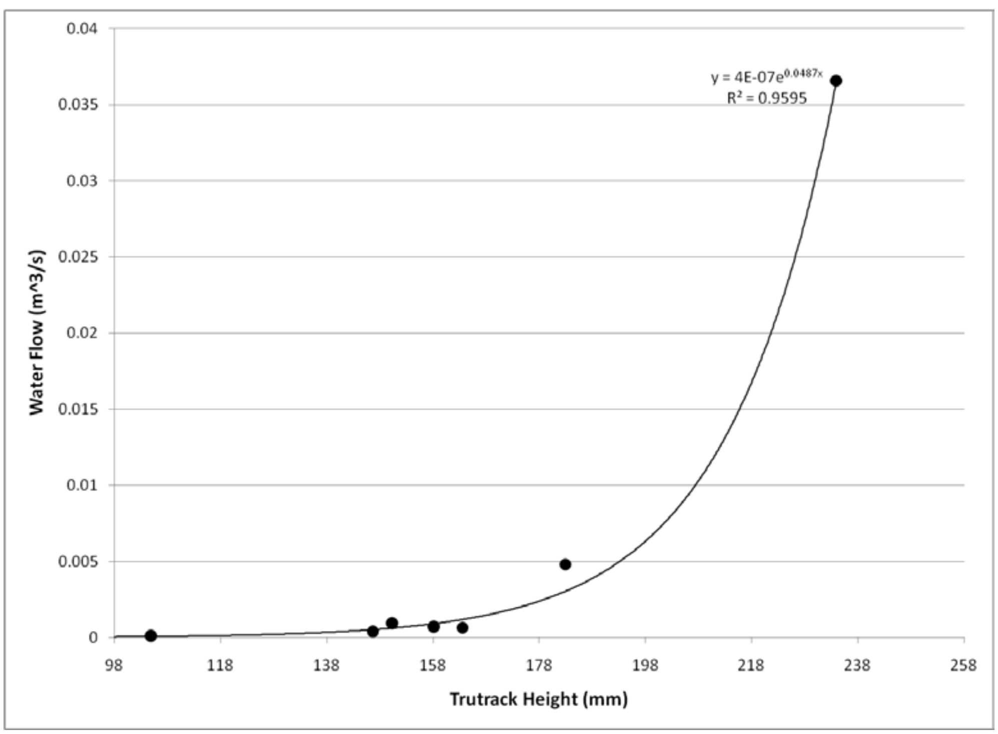


Figure A 5. Ditch 7 rating curve.

APPENDIX B. Examples of Intra-storm *E. coli* Patterns

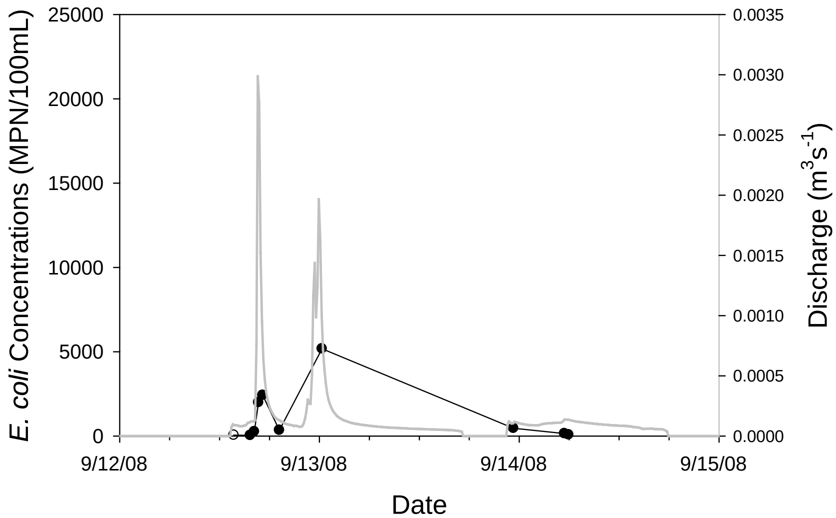


Figure B 1. Example of peak *E. coli* concentrations occurring during the peak flows in Ditch 1.

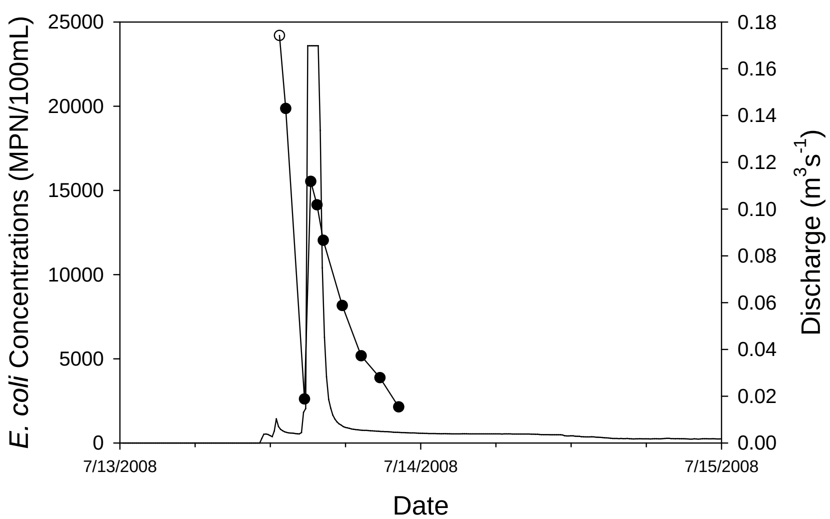


Figure B 2. Example of the first flush effect in Ditch 5.

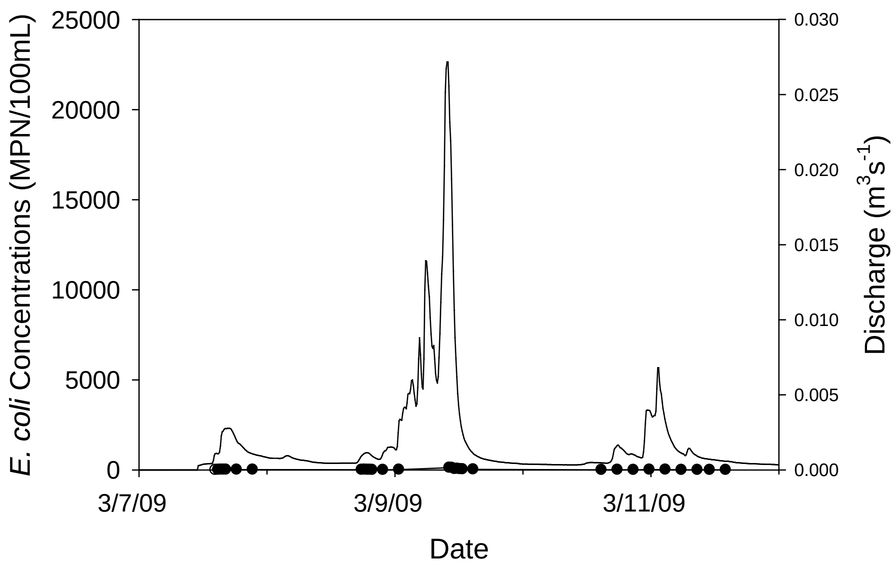


Figure B 3. Example of a peak in flow with no corresponding peak in *E. coli* concentrations in Ditch 2 during a spring runoff event.

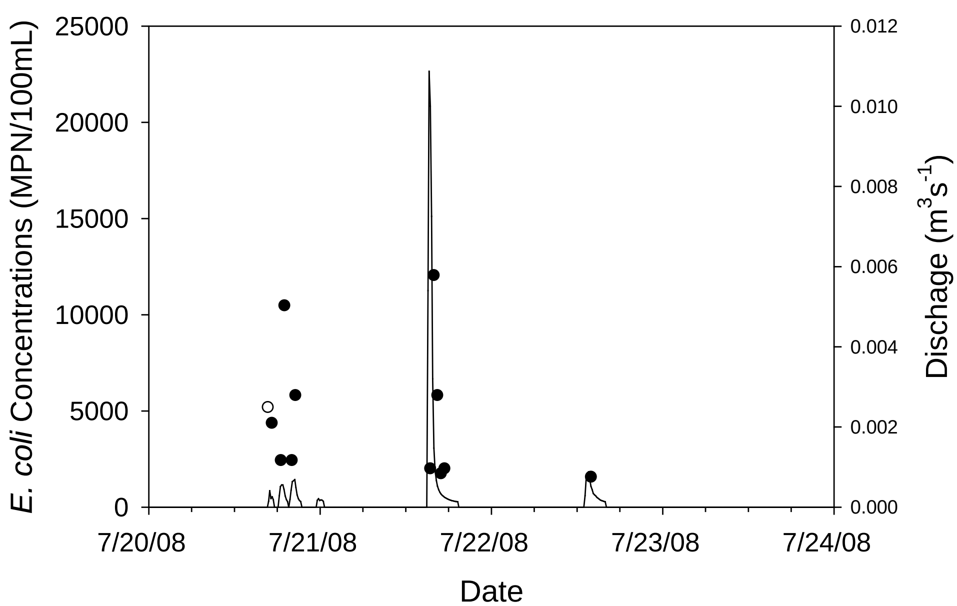


Figure B 4. Example of the flashiness of Ditch 3 during a summer storm.

APPENDIX C: Individual Ditch Discharge and E. coli and Total Suspended Solids Concentrations

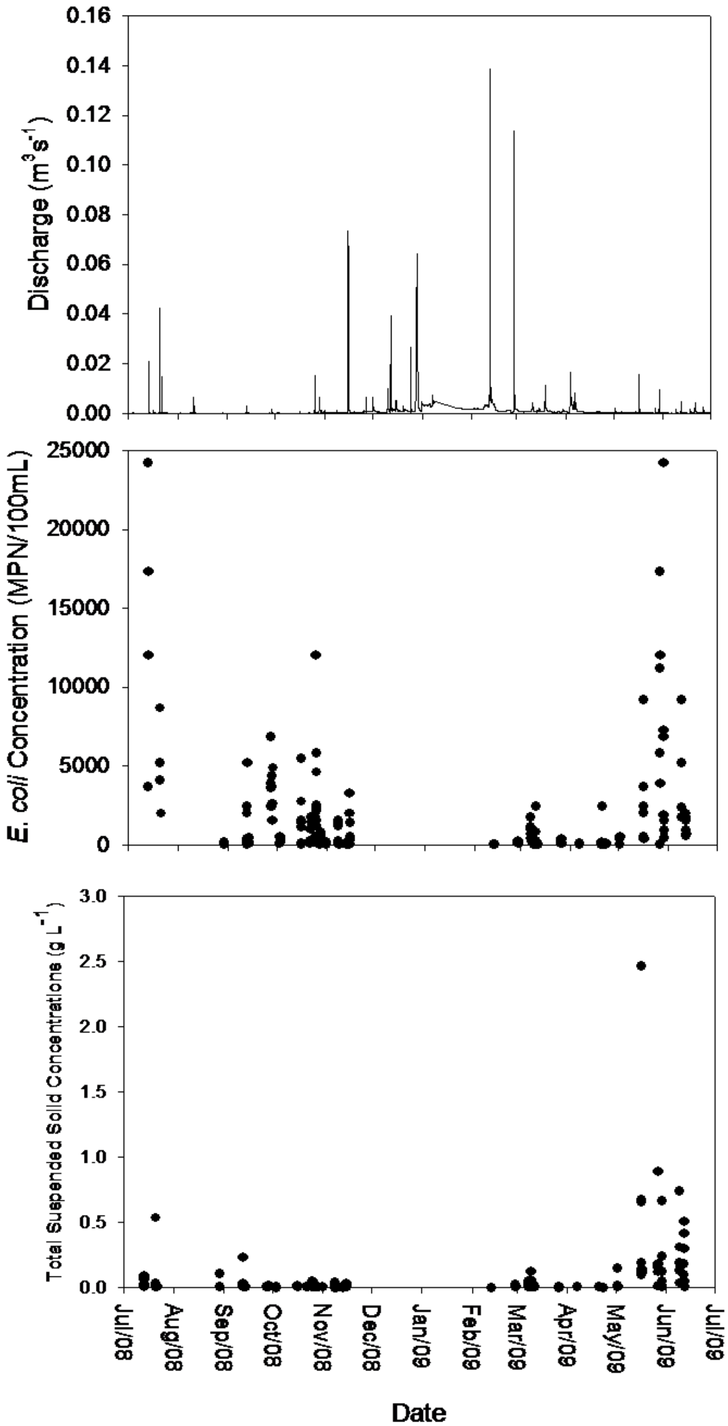


Figure C 1. Ditch 1, a forest roadside ditch, discharge, *E. coli* and total suspended solids concentration for entire study period.

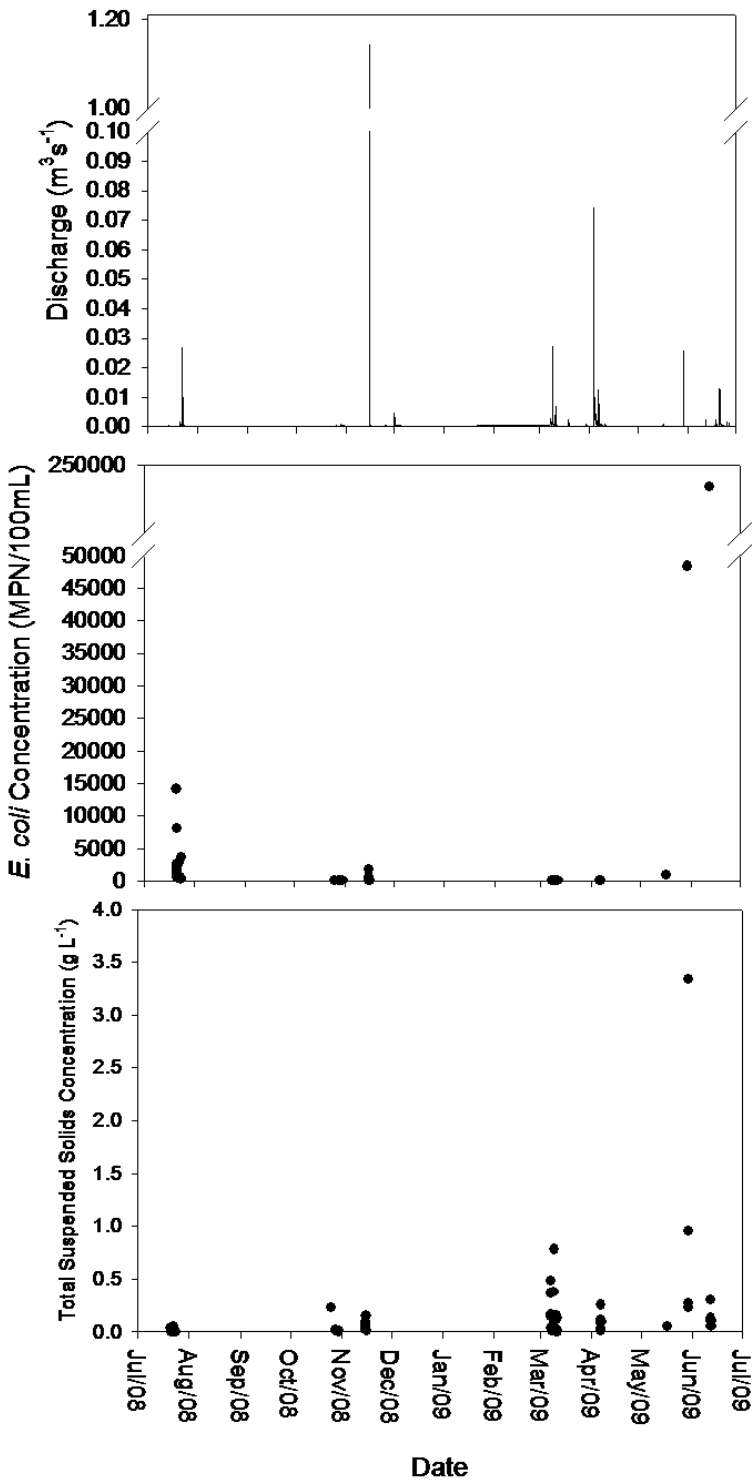


Figure C 2. Ditch 2, an agricultural roadside ditch, *E. coli* and total suspended solids concentrations and discharge over the entire study period.

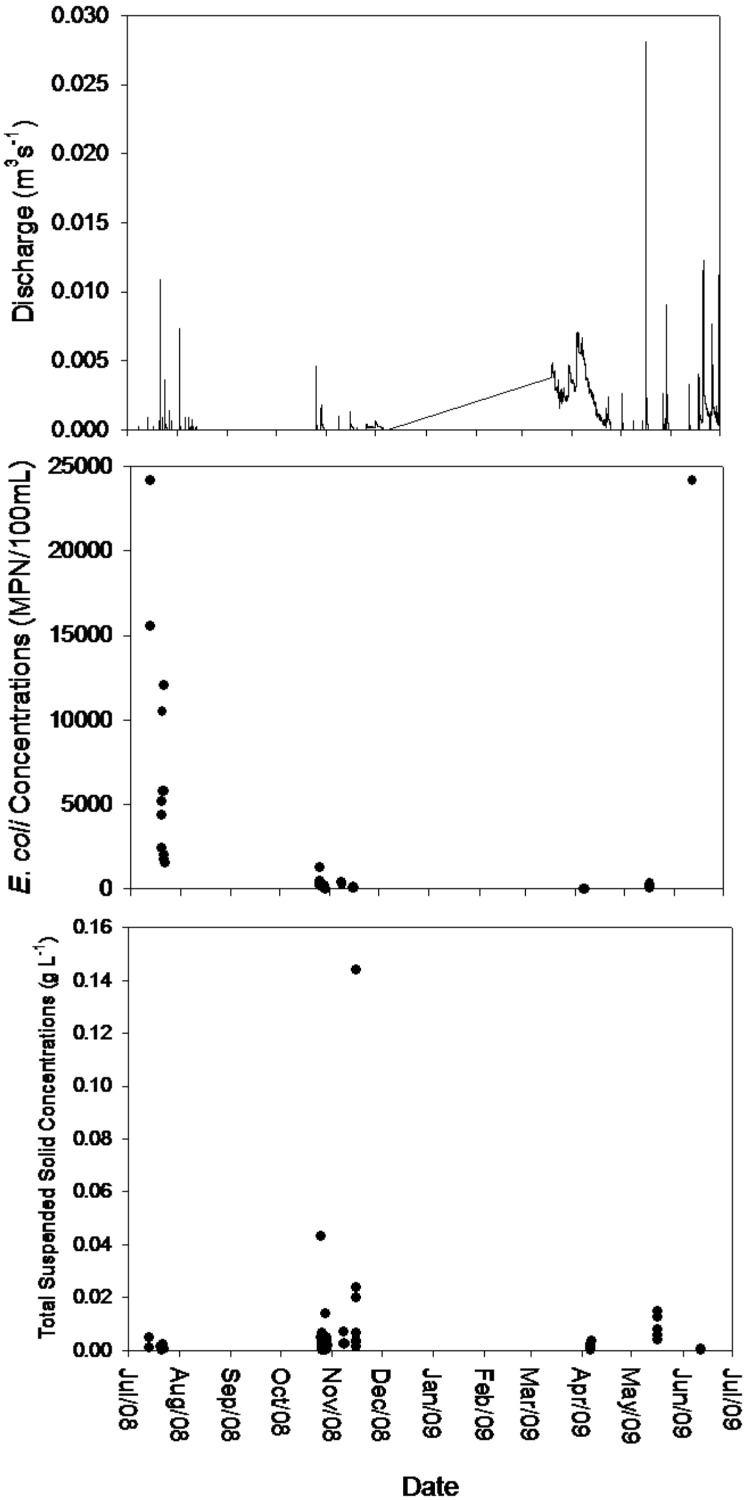


Figure C 3. Ditch 3, an agricultural roadside ditch, *E. coli* and total suspended solids concentrations and discharge over the entire study period.

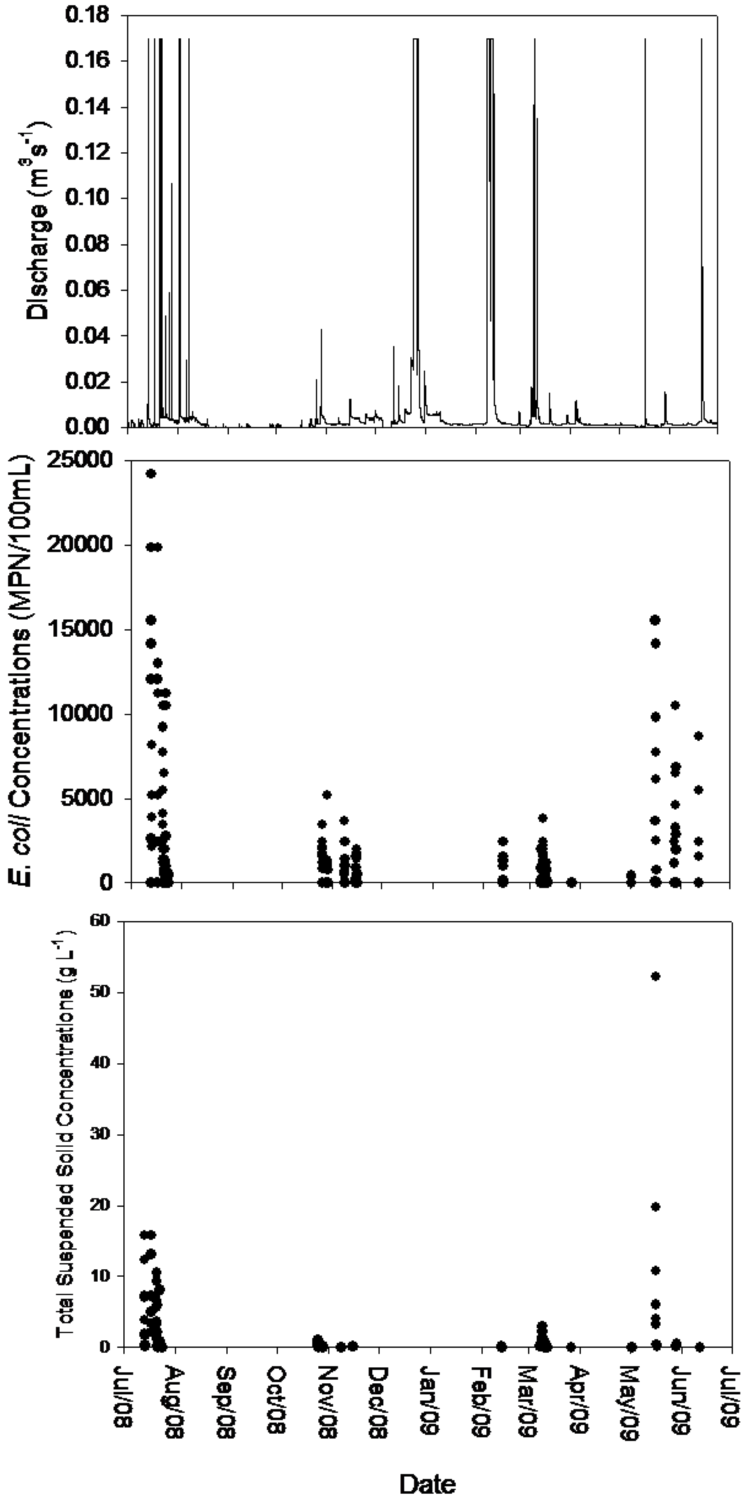


Figure C 4. Ditch 5, an agricultural roadside ditch, *E. coli* and total suspended solids concentrations and discharge over the entire period.

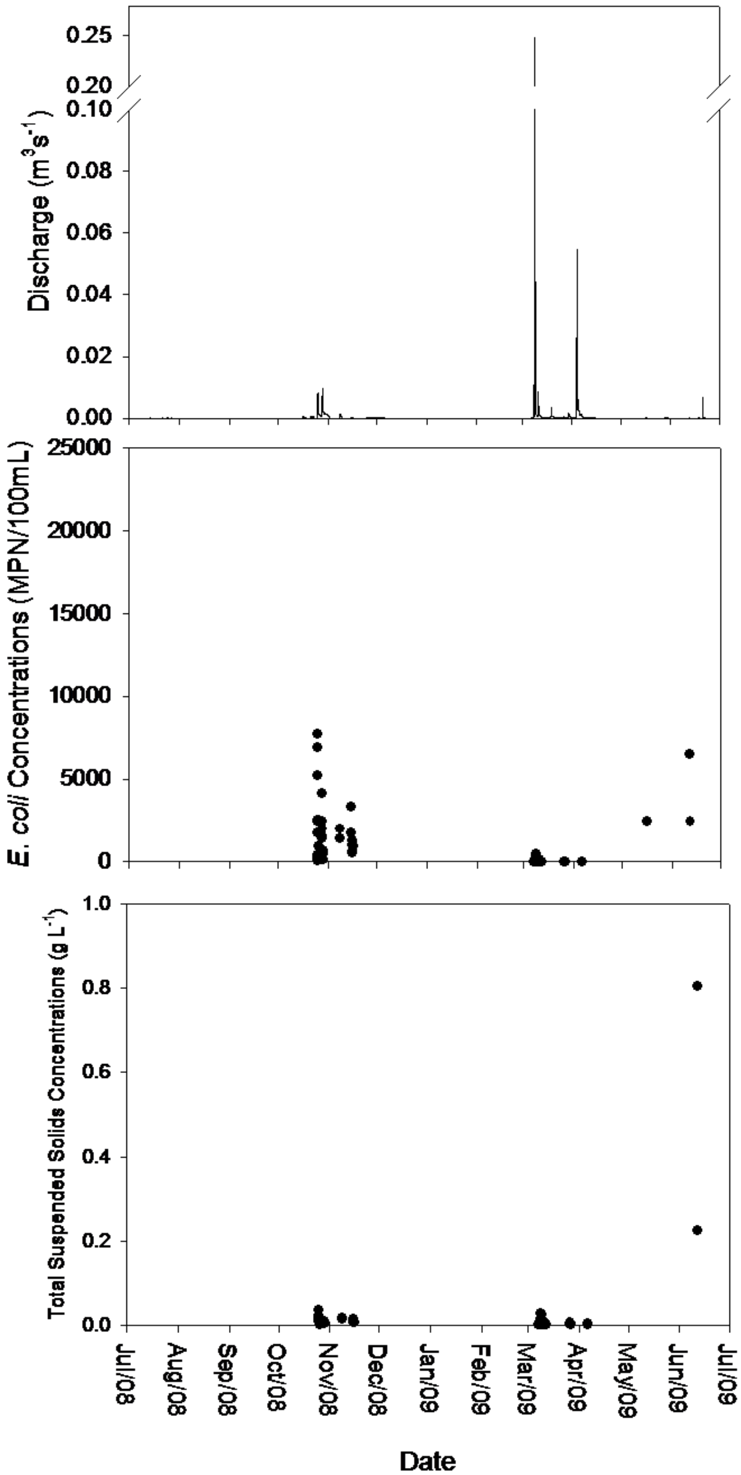


Figure C 5. Ditch 7, a forest roadside ditch, *E. coli* and total suspended solids concentrations over the entire study period.

APPENDIX D: Regression analysis of *E. coli* by roadside ditch discharge

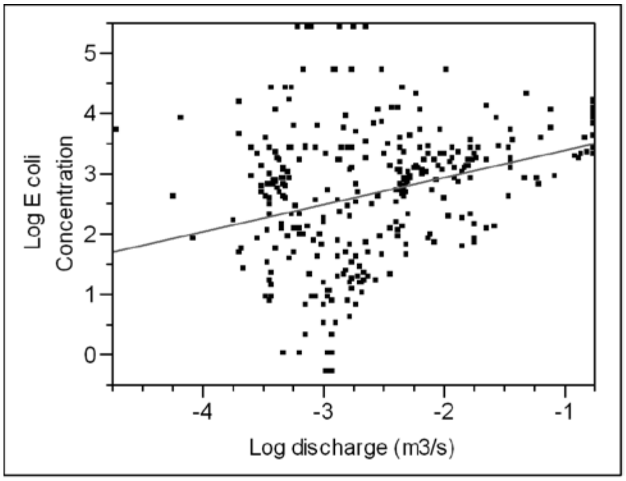


Figure D 1. Agriculture overall regression analysis between ditch discharge and *E. coli* concentrations (Log *E. coli* Concentration (MPN/100mL) =3.85 + 0.46xLog discharge (m3/s), p<.0001).

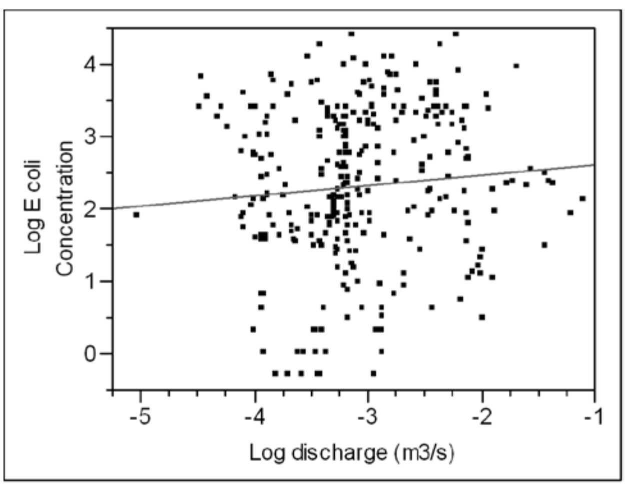


Figure D 2. Forest overall regression analysis between ditch discharge and *E. coli* concentrations (Log *E. coli* Concentration (MPN/100mL) =2.76 + 0.14xLog discharge (m3/s), p=0.1347).

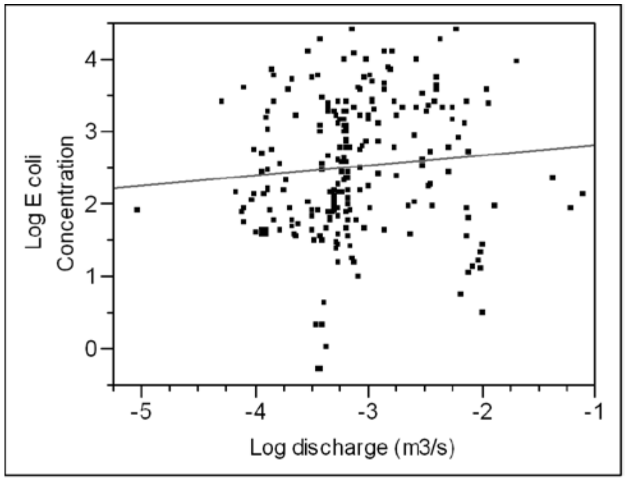


Figure D 3. Ditch 1 overall regression analysis between ditch discharge and *E. coli* concentrations (Log *E. coli* Concentration (MPN/100mL) = 2.93 + 0.14xLog discharge (m3/s), p=0.1880).

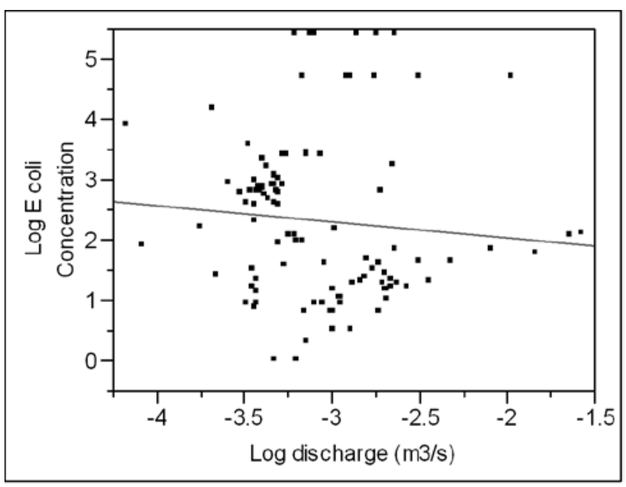


Figure D 4. Ditch 2 overall regression analysis between ditch discharge and *E. coli* concentrations (Log *E. coli* Concentration (MPN/100mL) = 1.51 - 0.26xLog discharge (m3/s), p=0.3747).

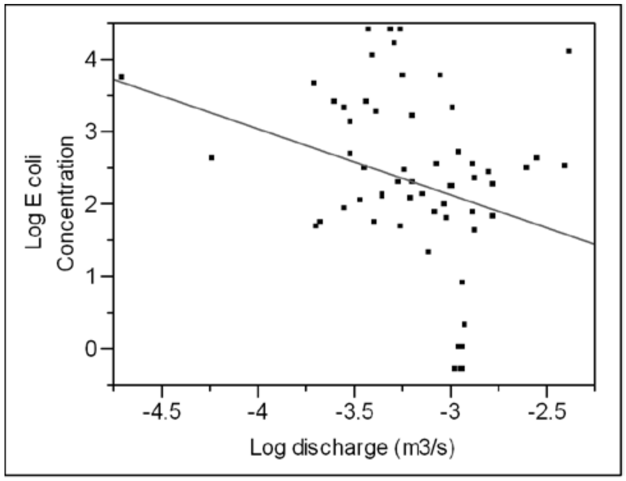


Figure D 5. Ditch 3 overall regression analysis between ditch discharge and *E. coli* concentrations (Log *E. coli* Concentration (MPN/100mL) = -0.59 - 0.91xLog discharge (m3/s), p=0.0246).

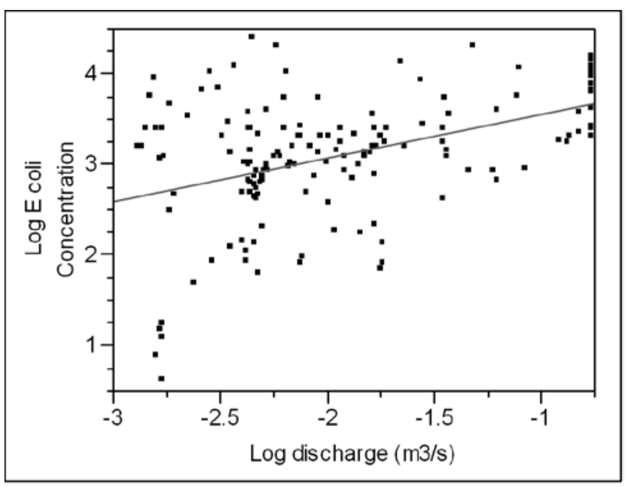


Figure D 6. Ditch 5 overall regression analysis between ditch discharge and *E. coli* concentrations (Log *E. coli* Concentration (MPN/100mL) = 4.06 + 0.49xLog discharge (m3/s), p<.0001).

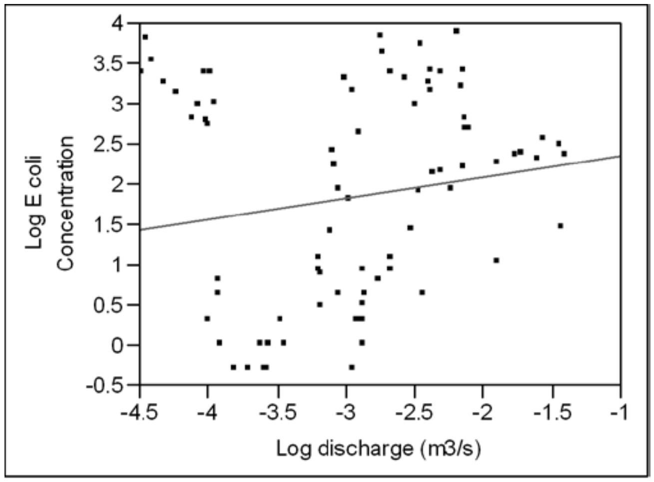


Figure D 7. Ditch 7 overall regression analysis between ditch discharge and *E. coli* concentrations (Log *E. coli* Concentration (MPN/100mL) = 2.62 + 0.26xLog discharge (m3/s), p=0.1474).

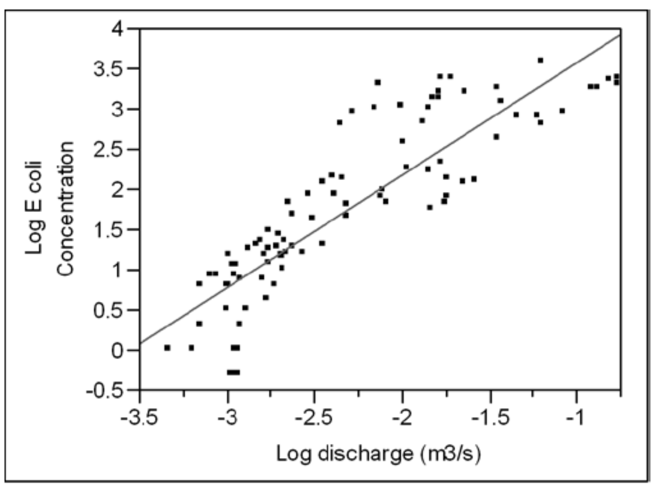


Figure D 8. Agriculture spring regression analysis between ditch discharge and *E. coli* concentrations (Log *E. coli* Concentration (MPN/100mL) = 4.97 + 1.40xLog discharge (m3/s), p<.0001).

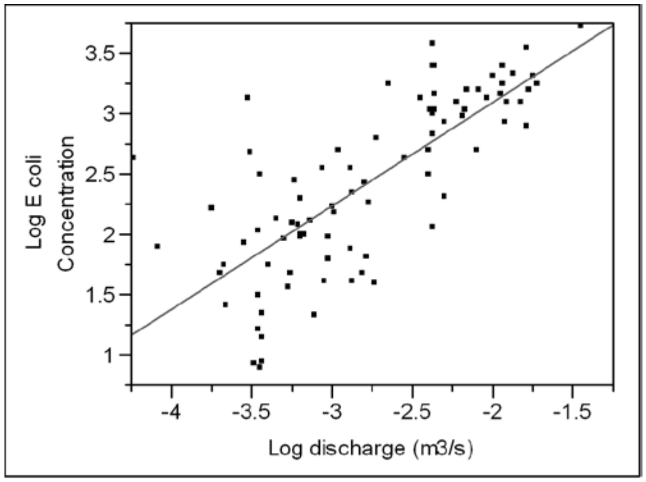


Figure D 9. Agriculture fall regression analysis between ditch discharge and *E. coli* concentrations (Log *E. coli* Concentration (MPN/100mL) = 4.80 + 0.86xLog discharge (m3/s), p<.0001).

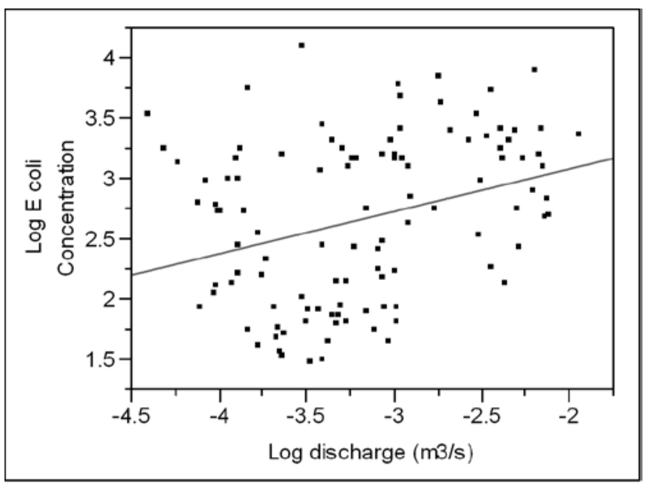


Figure D 10. Forest fall regression analysis between ditch discharge and *E. coli* concentrations (Log *E. coli* Concentration (MPN/100mL) = 3.77 + 0.35xLog discharge (m3/s), p=0.0008).

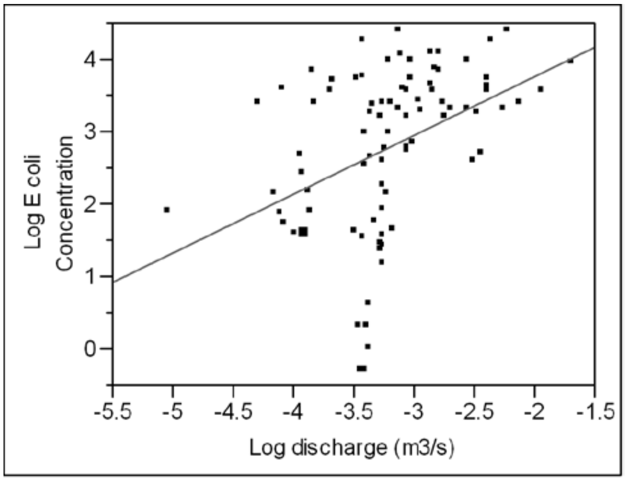


Figure D 11. Ditch 1 summer season regression analysis between ditch discharge and *E. coli* concentrations (Log *E. coli* Concentration (MPN/100mL) = 5.39 + 0.81xLog discharge (m3/s), p=0.0001).

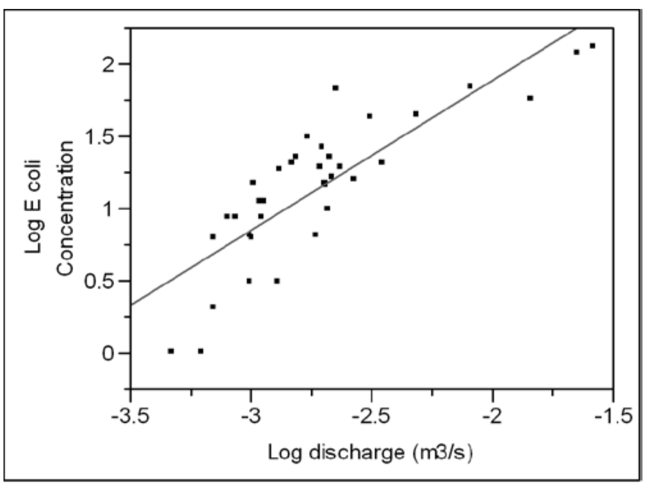


Figure D 12. Ditch 2 spring season regression analysis between ditch discharge and *E. coli* concentrations (Log *E. coli* Concentration (MPN/100mL) = 3.95 + 1.03xLog discharge (m3/s), p<.0001).

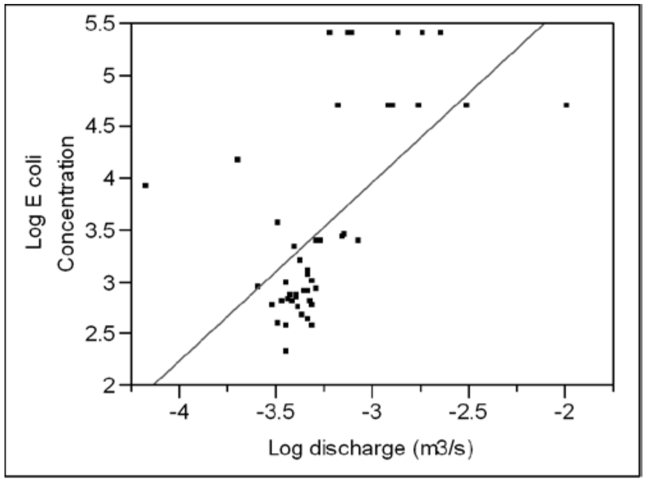


Figure D 13. Ditch 2 summer season regression analysis between ditch discharge and *E. coli* concentrations (Log *E. coli* Concentration (MPN/100mL) = 9.14+ 1.72xLog discharge (m3/s), p=<.0001).

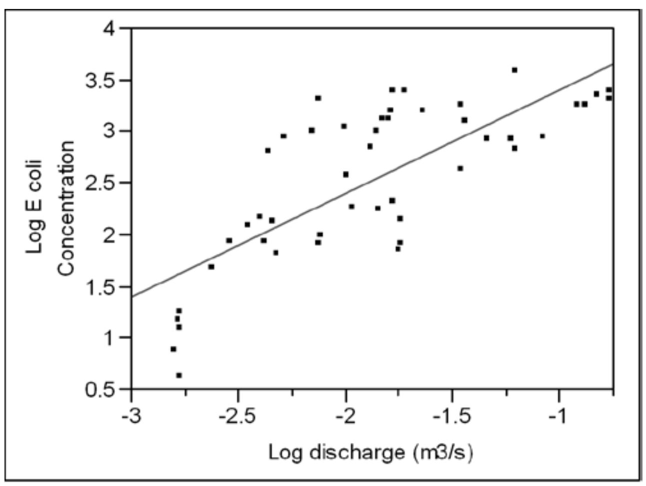


Figure D 14. Ditch 5 spring season regression analysis between ditch discharge and *E. coli* concentrations (Log *E. coli* Concentration (MPN/100mL) = 4.39 + 1.00xLog discharge (m3/s), p<.0001).

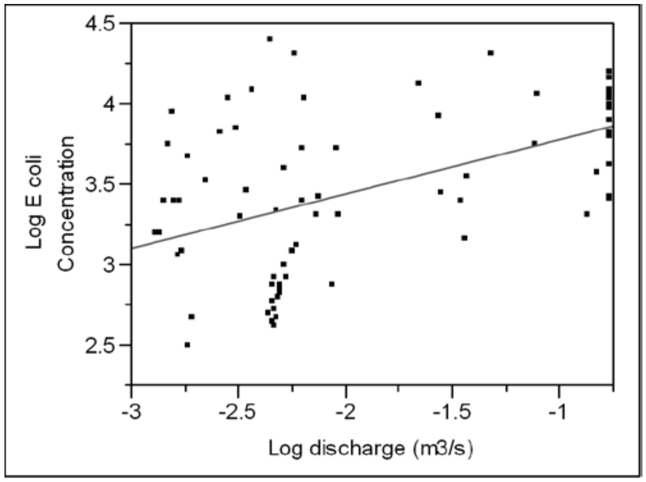


Figure D 15. Ditch 5 summer season regression analysis between ditch discharge and *E. coli* concentrations (Log *E. coli* Concentration (MPN/100mL) = 4.11 + 0.34xLog discharge (m3/s), p<.0001).

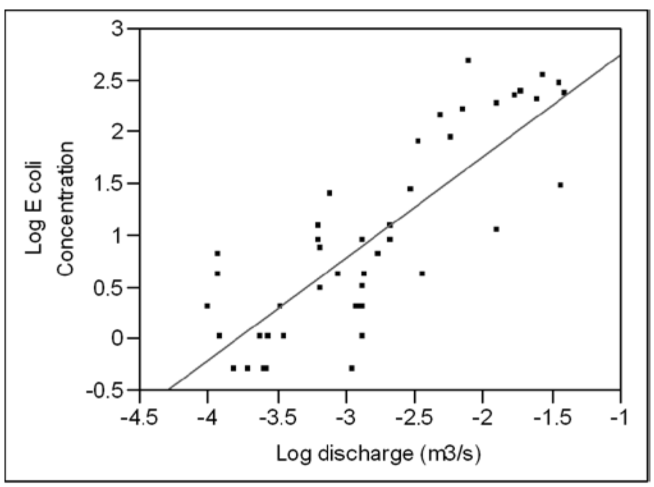


Figure D 16. Ditch 7 spring season regression analysis between ditch discharge and *E. coli* concentrations (Log *E. coli* Concentration (MPN/100mL) =3.72 + 0.98xLog discharge (m3/s), p<.0001).

APPENDIX E: Regression analysis between *E. coli* and total suspended solids concentrations.

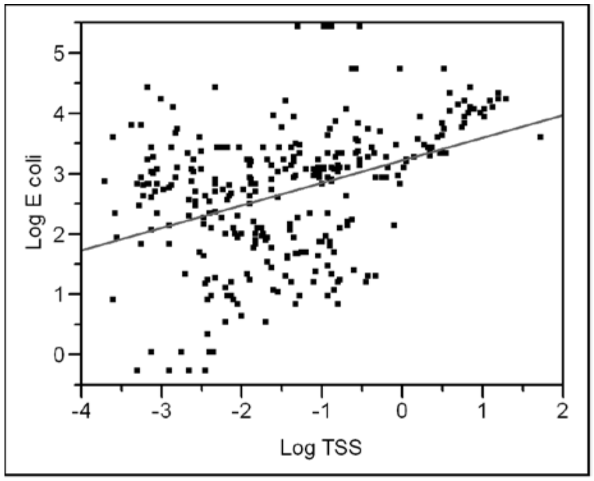


Figure E 1. Agriculture overall regression analysis between total suspended solids concentrations and *E. coli* (Log *E. coli* concentrations (MPN/100mL) = 3.23 + 0.38xLog TSS (g/L), p<.0001).

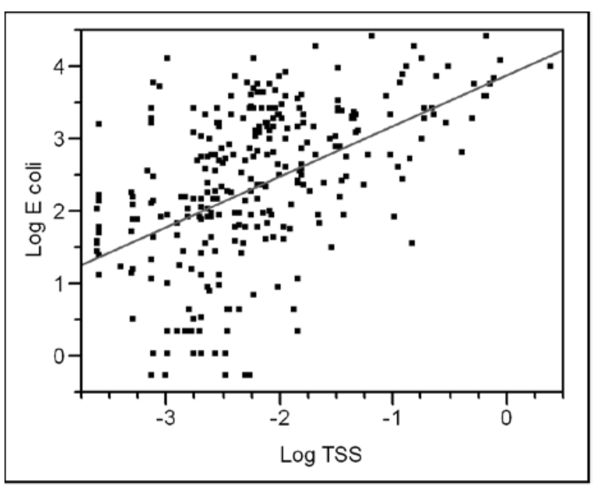


Figure E 2. Forest overall regression analysis between total suspended solids concentrations and *E. coli* (Log *E. coli* concentrations (MPN/100mL) =3.87 + 0.70xLog TSS (g/L), p<.0001).

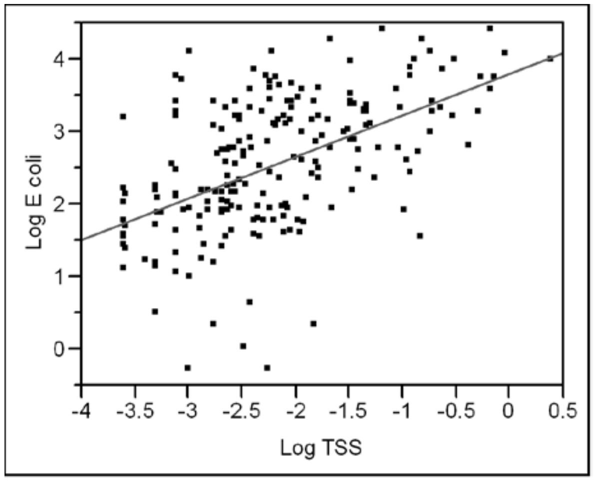


Figure E 3. Ditch 1 overall regression analysis between total suspended solids concentrations and *E. coli* (Log *E. coli* concentrations (MPN/100mL) = 3.79 + 0.57xLog TSS (g/L), p<.0001).

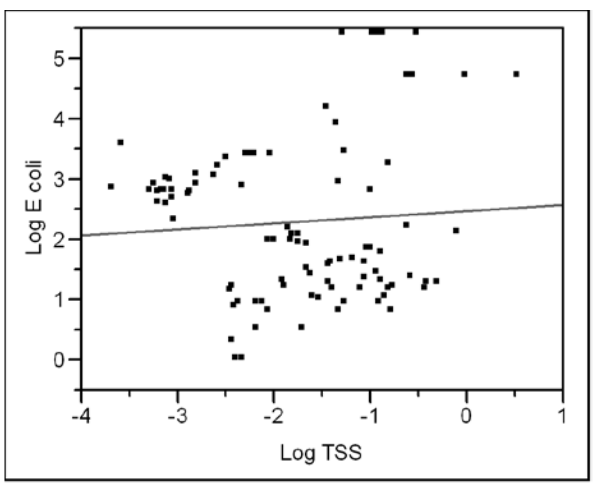


Figure E 4. Ditch 2 overall regression analysis between total suspended solids concentrations and *E. coli* (Log *E. coli* concentrations (MPN/100mL) =2.47 + 0.10xLog TSS (g/L), p=0.4968).

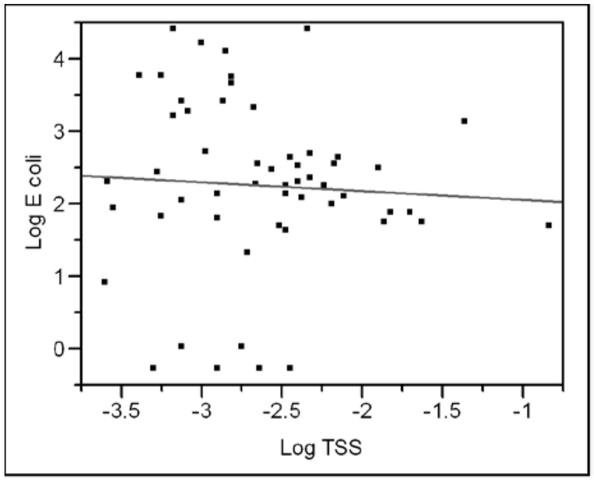


Figure E 5. Ditch 3 overall regression analysis between total suspended solids concentrations and *E. coli* (Log *E. coli* concentrations (MPN/100mL) = 1.94 - 0.12xLog TSS (g/L), p=0.6836).

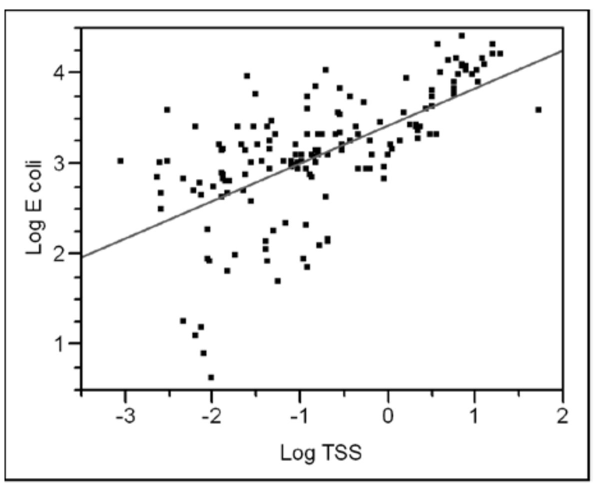


Figure E 6. Ditch 5 overall regression analysis between total suspended solids concentrations and *E. coli* (Log *E. coli* concentrations (MPN/100mL) =3.43 + 0.41xLog TSS(g/L), p<.0001).

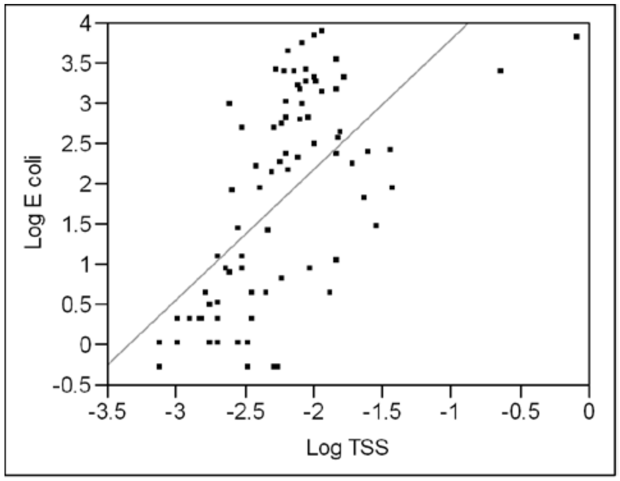


Figure E 7. Ditch 7 overall regression analysis between total suspended solids concentrations and *E. coli* (Log *E. coli* concentrations (MPN/100mL) =5.42 + 1.62xLog TSS (g/L), p<.0001).

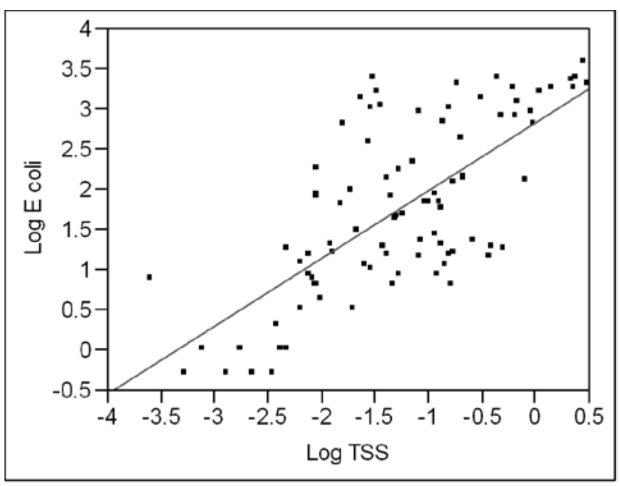


Figure E 8. Agriculture spring regression analysis between total suspended solids concentrations and *E. coli* (Log *E. coli* concentrations (MPN/100mL) = 2.82 + 0.84xLog TSS (g/L), p<.0001).

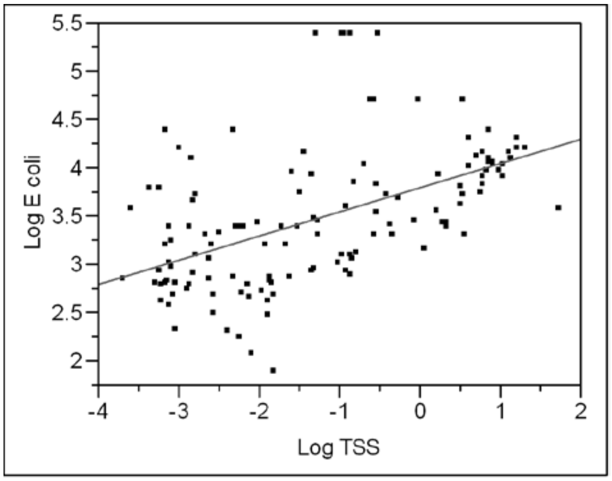


Figure E 9. Agriculture summer regression analysis between total suspended solids concentrations and *E. coli* (Log *E. coli* concentrations (MPN/100mL) =3.80 + 0.25xLog TSS (g/L), p<.0001).

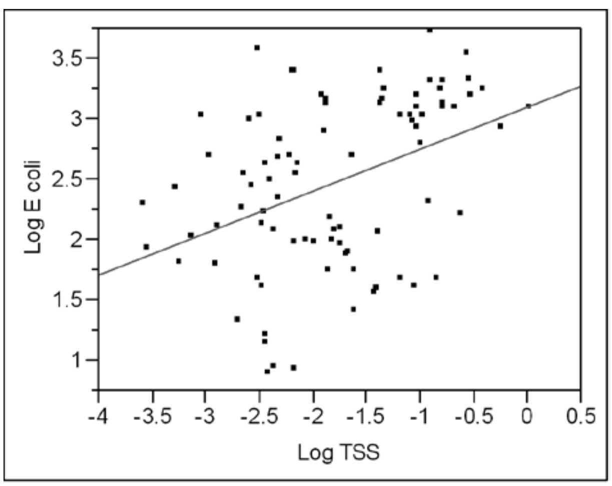


Figure E 10. Agriculture fall regression analysis between total suspended solids concentrations and *E. coli* (Log *E. coli* concentrations (MPN/100mL) =3.09 + 0.35xLog TSS (g/L), p<.0001).

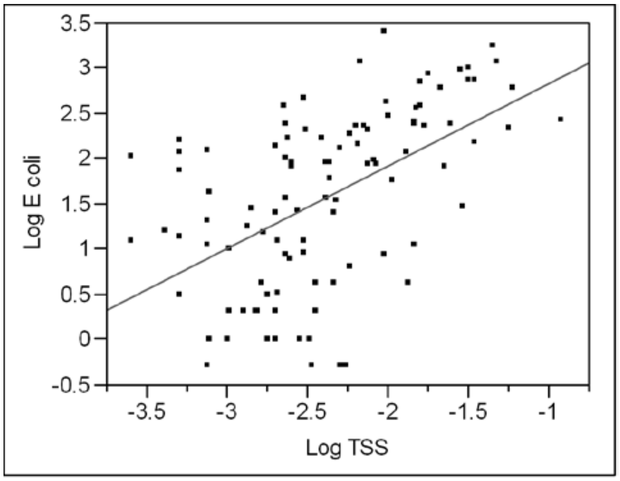


Figure E 11. Forest spring regression analysis between total suspended solids concentrations and *E. coli* (Log *E. coli* concentrations (MPN/100mL) = 3.73 + 0.91xLog TSS (g/L), p<.0001).

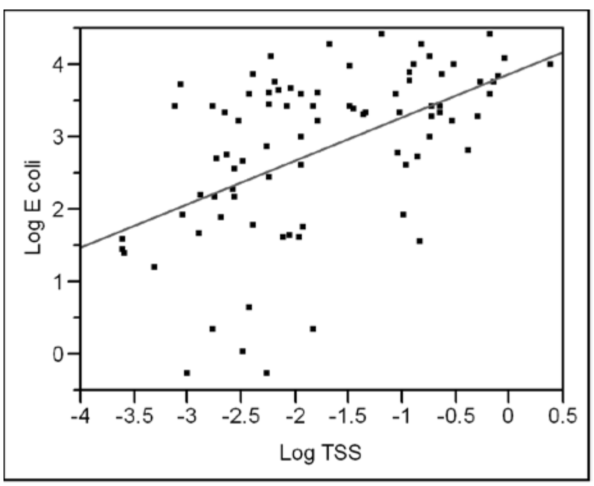


Figure E 12. Forest summer regression analysis between total suspended solids concentrations and *E. coli* (Log *E. coli* concentrations (MPN/100mL) = 3.87 + 0.60xLog TSS (g/L), p<.0001).

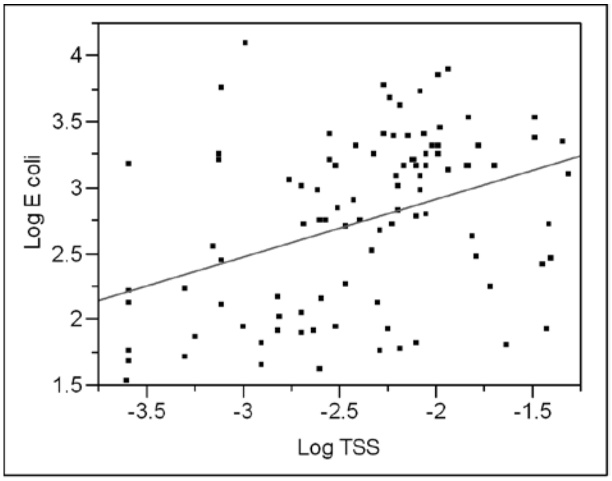


Figure E 13. Forest fall regression analysis between total suspended solids concentrations and *E. coli* (Log *E. coli* concentrations (MPN/100mL) = 3.79 + 0.44xLog TSS (g/L), p=0.0001).

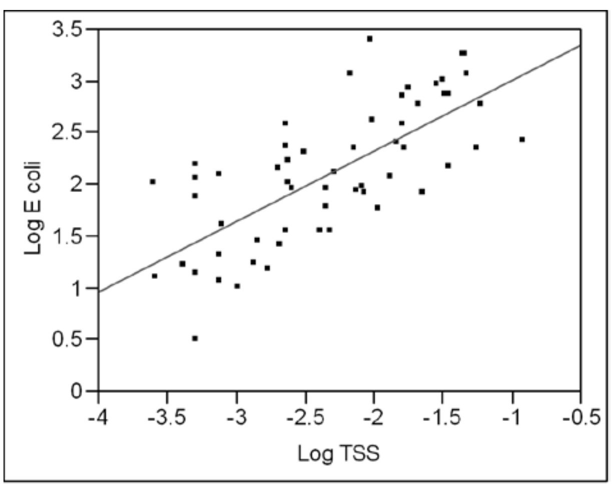


Figure E 14. Ditch 1 spring regression analysis between total suspended solids concentrations and *E. coli* (Log *E. coli* concentrations (MPN/100mL) = 3.68 + 0.68xLog TSS (g/L), p<.0001).

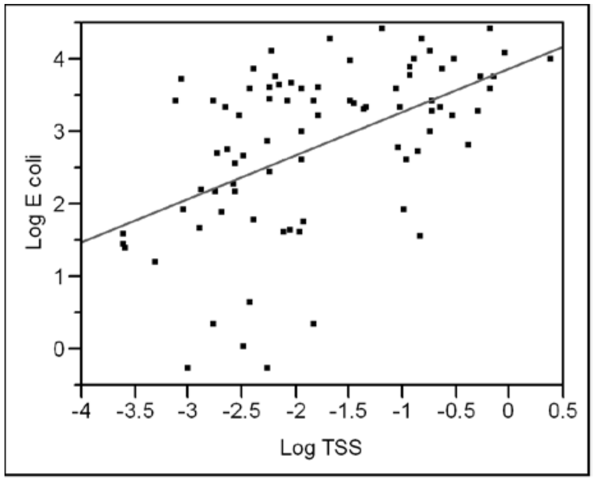


Figure E 15. Ditch 1 summer regression analysis between total suspended solids concentrations and *E. coli* (Log *E. coli* concentrations (MPN/100mL) =3.88 + 0.60xLog TSS (g/L), p<.0001).

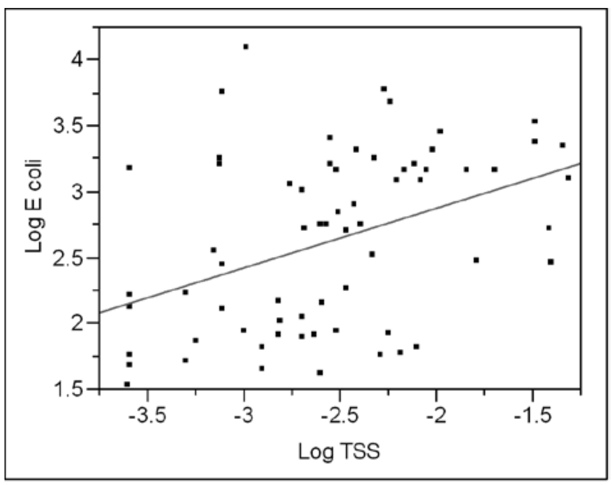


Figure E 16. Ditch 1 fall regression analysis between total suspended solids concentrations and *E. coli* (Log *E. coli* concentrations (MPN/100mL) =3.78 + 0.45xLog TSS (g/L), p=0.0007).

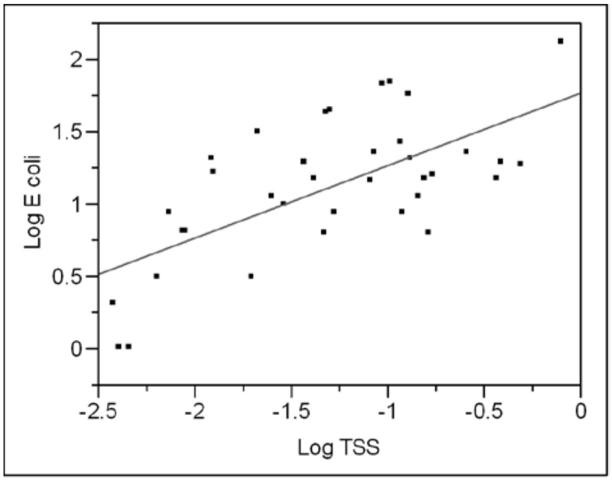


Figure E 17. Ditch 2 spring regression analysis between total suspended solids concentrations and *E. coli* (Log *E. coli* concentrations (MPN/100mL) =1.77+ 0.50xLog TSS (g/L), $p<.0001$).

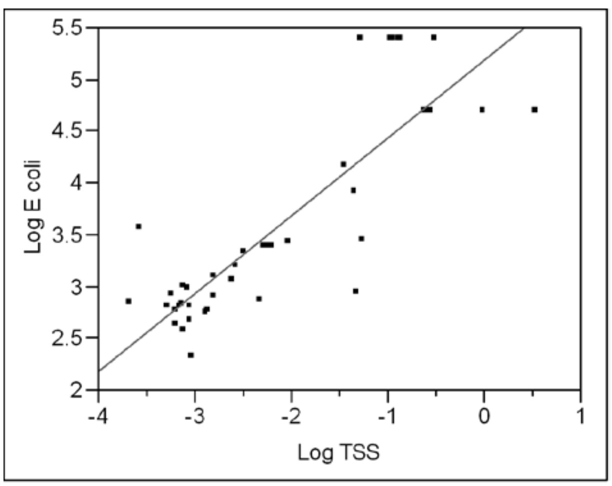


Figure E 18. Ditch 2 summer regression analysis between total suspended solids concentrations and *E. coli* (Log *E. coli* concentrations (MPN/100mL) = 5.19 + 0.75xLog TSS (g/L), $p=<.0001$).

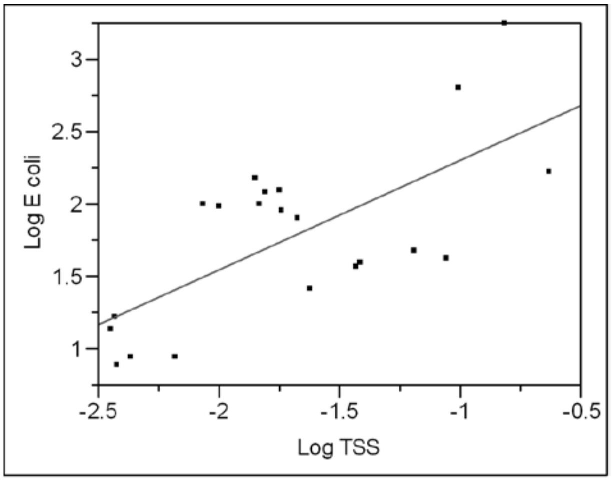


Figure E 19. Ditch 2 fall regression analysis between total suspended solids concentrations and *E. coli* (Log *E. coli* concentrations (MPN/100mL) = 3.06 + 0.76xLog TSS (g/L), p=0.0006).

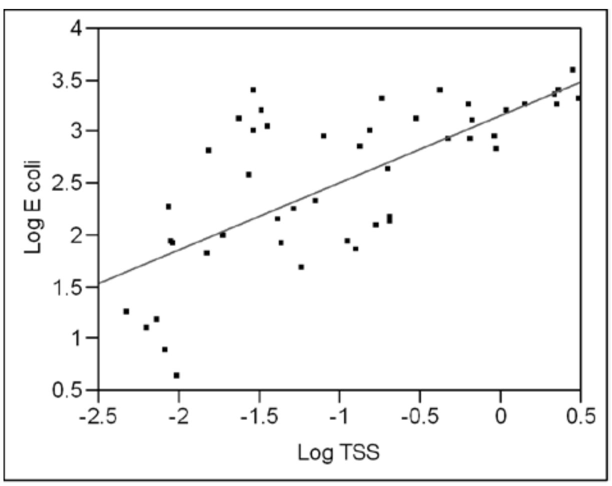


Figure E 20. Ditch 5 spring regression analysis between total suspended solids concentrations and *E. coli* (Log *E. coli* concentrations (MPN/100mL) =3.16 + 0.65xLog TSS (g/L), p=<.0001).

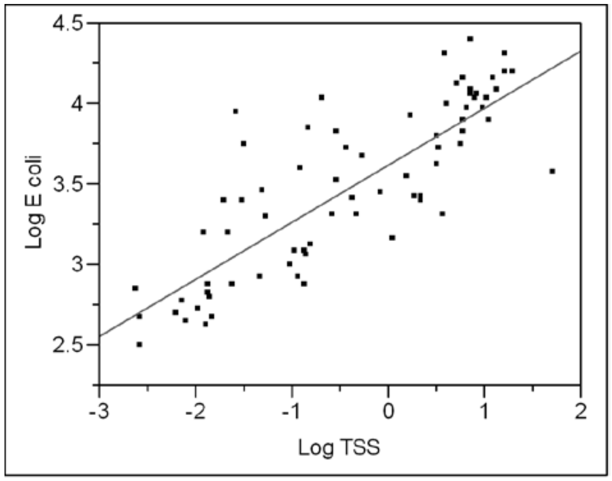


Figure E 21. Ditch 5 summer regression analysis between total suspended solids concentrations and *E. coli* (Log *E. coli* concentrations (MPN/100mL) =3.62 + 0.36xLog TSS (g/L), p<.0001).

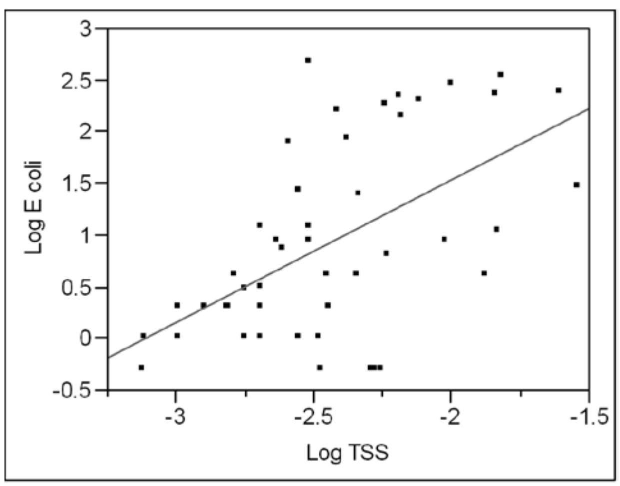


Figure E 22. Ditch 7 spring regression analysis between total suspended solids concentrations and *E. coli* (Log *E. coli* concentrations (MPN/100mL) =4.29 + 1.38xLog TSS (g/L), p<.0001).

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